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PREVALENCE AND CHARACTERISTICS OF *ESCHERICHIA COLI* ISOLATES HARBOURING SHIGA TOXIN GENES (*STX*) FROM ACUTE DIARRHOEAL PATIENTS IN DHAKA, BANGLADESH

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Abstract: Shiga toxin genes (*stx*) harbouring *Escherichia coli* (STEC) strains were isolated and identified from diarrhoeal patients visiting the Dhaka Hospital of ICDDR,B: Centre for Health and Population Research, Dhaka, Bangladesh. Of the 189 *E. coli* strains isolated from 775 diarrhoeal stool specimens, 19 harboured *stx1*, and one isolate was revealed to have amplicons for both *stx1* and *stx2* by a PCR assay. Sequence analysis of the 349-bp *stx1* from representative isolates revealed 100% homology with the sequence of *stx1* available in the GenBank. Among the *stx1* positive isolates, two harboured the *eae* but none were positive for *hlyA, katP, etpD* or *saa* genes. Fifteen of the 20 *stx* positive strains could be categorized into 13 non-O157 serogroups while 4 were untypable and one was a rough strain. Most of the STEC strains were resistant to ampicillin, cephalothin, co-trimoxazole, tetracycline, and nalidixic acid. In the Vero cell assay, all the strains were negative for expression of Shiga toxin (Stx). Randomly amplified polymorphic DNA (RAPD) PCR analysis demonstrated genetic diversity. This is one of the first reports to show the presence of STEC in diarrhoeal patients in Bangladesh.
Key Words: Diarrhoea, STEC, PCR, Shiga toxin gene, Genetic diversity

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC), a distinct class of pathogenic *E. coli,* can cause a spectrum of human illnesses ranging from asymptomatic carriage to severe bloody-diarrhoea termed haemorrhagic colitis (HC), as well as life-threatening sequels such as haemolytic uremic syndrome (HUS) which has a case fatality rate of up to 5% [1, 2]. STEC has gained immense clinical importance since being recognized in food-borne outbreaks in Oregon and Michigan in 1982 [3], and subsequently it has emerged as a major public health problem in many developed countries. The rate of morbidity and mortality due to Shiga toxin-producing *E. coli* (STEC) has highlighted the threat these organisms pose to public health. The ability of STEC to cause serious disease in humans is related to the production of one or more Shiga toxins (Stx1, Stx2 or their variants), which inhibit protein synthesis of host cells leading to death [4, 5]. Stx1, Stx2 and their variants are immunologically non-cross reactive and are encoded by alleles in the genome of temperate, lambdoid bacteriophages that remain integrated in the *E. coli* chromosome [6]. Stx1 is virtually identical to Shiga toxin produced by *Shigella dysenteriae* 1, while Stx2 has only 56% identity to Stx1 [7].

The pathogenesis of STEC is triggered by a set of genes carried in the chromosome that includes a 35.5 kb pathogenicity island, termed locus of enterocyte effacement (LEE). The characteristic attaching and effacing (A/E) lesion caused by most of the STEC depends on the activity of multiple genes in the LEE including the type III secretion system and on the initiation of signal transduction events [8]. The *eae* gene is responsible for attaching to translo-

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cated intimin receptor (Tir). Some of the STEC strains harbour a 97 kb plasmid (pO157), encoding possible additional virulence genes such as *hlyA* (EHEC-HlyA) that acts as a pore-forming cytolysin on eukaryotic cells, the bifunctional catalase peroxidase (KatP), the *etpD* gene cluster that probably encodes a type II secretion pathway, and the secreted serine protease (*EspP*) [9-13]. The *saa* gene (STEC autoagglutination adhesin) encodes a novel outer membrane protein and is carried on megaplasmid of LEE-negative STEC strains, which acts as an autoagglutinating adhesin [14].

About 250 non-O157 STEC serotypes have been reported to be associated with diarrhoea, HUS and other afflictions [15, http://www.Microbionet.com.au/frames/feature/vetc/brief01.html]. In the USA, non-O157 serotypes are detected at regular intervals but lack many important virulence genes [16]. Among STEC, the serotype O157:H7 is epidemiologically significant worldwide (http:www.who.int/emcdocuments/zoonooses/whocsraph988.c.hlml). STEC infection has posed a much greater problem to developed countries than to developing countries. The low incidence of STEC in developing countries might be attributable to the complexity of recognising emerging variants [17, 18]. Though STEC has been isolated from humans and cattle in India [19], less is known about STEC in Bangladesh. An earlier study observed the presence of SLT-I and SLT-II positive *E. coli* isolates in the paediatric population in Bangladesh [20]. This study was conducted to determine the significance of STEC- related diarrhoea in the Bangladeshi setting. As part of the active surveillance, we conducted a bacteriological analysis of faecal specimens from diarrheal patients visiting the Dhaka Hospital of ICDDR, B over a period of 5 months.

MATERIALS AND METHODS

Sample collection and isolation of *E. coli*

Faecal specimens were collected from patients enrolled in the 2% systematic sampling of all patients visiting the Dhaka Hospital of ICDDR, B under its diarrhoeal disease surveillance system, between July and November 2002. Information such as age, sex, and clinical symptoms (type and duration of diarrhoea, dehydration status, and presence of fever and other symptoms) was collected using a standard questionnaire. All the 775 faecal specimens collected from patients in sterile containers were subjected to macroscopic inspection for presence of blood and mucus, and for isolation of enteric bacterial pathogens using standard laboratory methods [21]. From each faecal specimen, three representative lactose-fermenting colonies growing on MacConkey agar (Difco, Detroit, USA) were isolated and identified [21]. The ability of the *stx* harbouring isolates to ferment sorbitol was assessed by streaking of the strains on sorbitol MacConkey Agar (Difco) plates. O-serogroups of *E. coli* isolates were determined by slide agglutination using a commercially available antisera kit (Denka Seiken Co., Tokyo, Japan). All of the *E. coli* isolates were preserved at -80 ℃.

Drug susceptibility tests

Drug susceptibility was determined by the disc diffusion method using the following commercial disks (Hi Media, Mumbai, India), ampicillin (10 μg), chloramphenicol (30 μg), co-trimoxazole (25 μg), ciprofloxacin (5 μg), gentamicin (10 μg), neomycin (30 μg), nalidixic acid (30 μg), norfloxacin (10 μg), streptomycin (10 μg), tetracycline (30 μg), cephalothin (30 μg), amikacin (30 μg), furazolidone (50 μg), kanamycin (30 μg) and ceftriaxone (30 μg). The *E. coli* ATCC 25922 strain sensitive to all the drugs was used as a quality control. The characterization of the isolates as susceptible, reduced susceptibility, or resistant was done as recommended by the National Committee for Clinical Laboratory Standards [22].

Screening for virulence genes

PCR for the detection of both chromosomal and plasmid virulence genes was performed using a thermal cycler (Applied Biosystems, Foster City, Calif, USA) in a total volume of 25 μl. The reaction mixture contained 5 μl of culture lysate, 2.5 mM of each dNTP, 30 μM of each primer, 2.5 μl of 10 X PCR Buffer, and 1 U of r-Taq DNA polymerase (Takara, Shuzo, Japan). The primer sequences of the targeted virulence genes and the PCR conditions are presented in Table 1. VTEC-3 (serotype O157:H7 harbouring *stx1* and *stx2* genes) and *E. coli* K-12 strain were used as positive and negative controls, respectively.

Southern hybridisation

The PCR products from 5 representative strains were separated in 1.5% agarose gel and transferred to a Nylon membrane by the capillary method, and the were UV cross-linked (GS Gene linker, Bio-Rad, Hercules, USA). The cloned *stx1* 905-bp DNA fragment from the recombinant plasmid pKTN501 was used as a probe after digestion with *Bam*HI and *Eco*RI. Hybridisation was done using the DIG-DNA Labelling and Detection Kit (Boehringer-Mannheim, Germany).

Sequencing of stx-PCR amplicon

The *stx1* gene was amplified using primers (EVT1 and EVT2). The PCR products were purified by QIA Quick PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced directly in an ABI310 automated sequencer (Applied Biosystems) using Big-dye terminator chemistry, and

Table 1. Polymerase Chain Reaction (PCR) primers and conditions used in the study

Primer	Nucleotide sequence of primers (5'-3')	Target	PCR conditions[a]			Amplicon (bp)	Reference
			Denaturing	Annealing	Extension		
EVT1/ EVT2	CAACACTGGATGATCTCAG CCCCCTCAACTGCTAATA	*stx1*	94 ℃, 60s	55 ℃, 60s[b]	72 ℃, 60s	349	48
EVS1/ EVC2	ATCAGTCGTCACTCACTGGT CTGCTGTCACAGTGACAAA	*stx2*	94 ℃, 30s	55 ℃, 60s[b]	72 ℃, 60s	110	48
hlyA1/ hlyA4	GGTGCAGCAGAAAAAGTTGTAG TCTCGCCTGATAGTGTTTGGTA	EHEC *hlyA*	94 ℃, 30s	57 ℃, 60s	72 ℃, 90s	1551	49
wkatB /wkatF	CTTCCTGTTCTGATTCTTCTGC AACTTATTTCTCGCATCATCC	*katP*	94 ℃, 30s	56 ℃, 60s	72 ℃, 90s	2125	50
D1/ D13R	CGTCAGGAGGATGTTCAG CGACTGCACCTGTTCCTGATTA	*etpD*	94 ℃, 30s	52 ℃, 60s	72 ℃, 70s	1062	51
EAE1/ EAE2	AAACAGGTGAAACTGTTGCC CTCTGCAGATTAACCTCTGC	*eae*	94 ℃, 60s	55 ℃, 90s	72 ℃, 90s	350	52
SAAF SAAR	CGTGATGAACAGGCTATTGC ATGGACATGCCTGTGGCAAC	*saa*	94 ℃, 30s	52 ℃, 60s	72 ℃, 40s	119	14

[a]Unless stated, PCR was done for 30 cycles.
[b]After 35 cycles, a final extension step of 10 min at 72 ℃ was performed.

Table 2. Clinical manifestation of patients infected with STEC.

Age Group (Y)	No. (%) STEC infected cases (n=20)	Presenting feature (%) (n=20)		
		Watery Stool	Vomiting	Dehydration
< 5yrs	11 (55)	11 (100)	8 (72)	4 (36)
> 5yrs	9 (45)	6 (67)	5 (55)	6 (67)

the obtained sequences were compared with published *stx1* gene sequences in the GenBank databases using the Blast search program of NCBI.

Vero cell assay

All of the STEC strains were cultured in L-broth (Difco) at 37 ℃ overnight with constant shaking and pelleted by centrifugation at 5000 rpm for 5 min at 4 ℃. Cell pellets were washed and sonicated with Handy Sonic (TOMY, Tokyo, Japan). After centrifugation, the supernatant and the cell lysate were filter-sterilized using 0.22 µm filters (Millex, Millipore, Bedford, USA) and used in the assay. The cytotoxic effect of the STEC strains was assayed on Vero-cells in 96-well flat bottom tissue culture plates (NUNC, Intermad, Denmark) as described elsewhere [23]. The cells were observed microscopically up to 72 hrs for cytotoxicity. VTEC-3 and DH5α were used as positive and negative controls, respectively.

RAPD Analysis

Molecular typing of *stx*-harbouring isolates was done by RAPD-PCR using a single primer 1247 (5'-AAGAGCC CGT-3') [19] in the Gene Amp PCR system 9700 (Applied Biosystems). The PCR mixture was made to a volume of 50 µl containing 100 ng of genomic DNA, 200 µM each dNTP, 30 pmol of primer, 5 µl of 10X PCR buffer, 3 mM $MgCl_2$ and 2.0 U of Taq DNA polymerase (Takara). After a hot start at 80 ℃ for 5 minutes, the DNA was subjected to 35 cycles of denaturing at 94 ℃ for 1 minute, annealing at 40 ℃ for 1 minute, and extension at 72 ℃ for 2 minutes. A final extension step was done for 10 minutes at 72 ℃. The ethidium bromide stained gels were digitised for comparison and to ascertain the clonal relationship between isolates.

Statistical analysis

To determine the statistical significance of diarrhoea among STEC infected patients in different age groups, we tested the data with Chi-square using 2x2 table in EpiInfo 2000, and Fischer Exact test was done to obtain the significance (p) value.

RESULTS

Clinical manifestation

No apparent predilection in the incidence of STEC was observed between patients of different age groups from whom *stx*-harbouring *E. coli* were isolated. When patients excreting *stx*-harbouring *E. coli* were stratified by age, we observed that watery diarrhoea was more common in the <5 year age group as compared to the >5 year of age group (p= 0.07). Though not significant, a similar trend was observed for vomiting and degree of dehydration (Table 2). Patients found to be carrying *stx*-harbouring *E. coli* were not co-infected with other enteric pathogens.

Table 3. Genotypic and phenotypic characteristics of the STEC strains isolated from hospitalized diarrhoea patients.

Strain	Serotype	Virulence gene								Phenotypic characteristics
		Chromosomal					Plasmid			
		stx$_1$	*stx*$_2$	*eae*	*saa*	*hlyA*	*katP*	*etpD*	SF[a]	Antibiogram
J1	O36a	+	-	-	-	-	-	-	-	AChFxKTCCiCoNaSN
J2	O127a	+	-	+	-	-	-	-	-	AChFxCoNa
J3	O6	+	+	-	-	-	-	-	-	AChFxTCCoNaS
J4	O63	+	-	-	-	-	-	-	-	AChCfTCoNaS
J5	O146	+	-	-	-	-	-	-	+	AChGCfFxKTCiCoNa
J6	O78	+	-	-	-	-	-	-	+	AChGFxTCoNaS
J7	ONT	+	-	+	-	-	-	-	+	ACh
J8	O44	+	-	-	-	-	-	-	+	AChCfTCCoNa
J9	O44	+	-	-	-	-	-	-	+	AChTCoNaS
J10	O8	+	-	-	-	-	-	-	+	AChCfTCoNaS
J11	Rough	+	-	-	-	-	-	-	+	AChCfTCCoNa
J12	O27	+	-	-	-	-	-	-	+	AChCfCCoNa
J13	O20	+	-	-	-	-	-	-	+	AChCfTCCiNa
J14	ONT	+	-	-	-	-	-	-	+	AChCfTCCiCoNa
J15	O25	+	-	-	-	-	-	-	+	AChCfKCoNa
J16	ONT	+	-	-	-	-	-	-	+	
J17	O44	+	-	-	-	-	-	-	+	AChTN
J18	O167	+	-	-	-	-	-	-	+	FxTCoS
J19	O15	+	-	-	-	-	-	-	+	AChTCoNa
J20	ONT	+	-	-	-	-	-	-	+	AChTCoS

[a]sorbitol fermentative

Abbreviations: A, ampicillin; Ch, cephalothin; G, gentamicin, Cf, ciprofloxacin, Fx, furazolidone; K, kanamycin; T, tetracycline; C, chloramphenicol; Ci, ceftriaxone; Co, co-trimoxazole; Na, nalidixic acid; S, streptomycin; N, neomycin.

Prevalence of STEC and characterization of the strains

During the study period, 775 faecal specimens were examined from diarrhoeal patients of various age groups. *E. coli* was cultured from 189 (24.4%) of the samples. Twenty *E. coli* isolates were positive (10.6%) in the *stx*-PCR assay indicating an overall incidence rate of 2.6%. All the *stx* harbouring strains were negative for O157 antiserum but belonged to 13 other O serogroups (Table 3). Among the 15 typable strains, O44 serogroup was common (20%) and 4 were not typable (ONT). All except 4 of the STEC strains were found to ferment sorbitol on MacConkey agar (Table 3).

Drug susceptibility

Most of the tested strains were resistant to ampicillin and cephalothin (90% each), co-trimoxazole (80%), tetracycline and nalidixic acid (75% each), ciprofloxacin (45%), streptomycin (40%), chloramphenicol (35%) and furazolidone (30%). The strain J16 was susceptible to all the tested drugs (Table 3). None of the isolates was resistant to amikacin or norfloxacin. As shown in Table 3, there was no common resistance pattern among the 20 strains tested.

Vero cell assay

In the Vero cell cytotoxic assay, except for VTEC-3 (positive control strain), none of the *stx* harbouring strains exhibited the characteristic cytotoxicity.

Detection of virulence genes and molecular typing

In the multiplex PCR assay, we identified 20 *E. coli* strains harbouring *stx*, 19 carrying the *stx1* and one carrying both *stx1* and *stx2* (Table 3). To confirm the authenticity of the PCR products, Southern hybridization was performed with DIG-labelled cloned probe from pKTN501. In addition, the amplified *stx1* gene of representative strains was sequenced and the sequence showed 100% homology with published *stx1* gene sequences. We identified two strains (J 2, J7) that had the *eae* (Table 3). None of the 20 *stx*-harbouring strains were positive for *hlyA, kat, etpD* or *saa* genes in PCR assays.

The *stx*-harbouring isolates were characterized by RAPD-PCR to analyse the genetic relatedness. All of the strains showed multiple amplicons with fragment sizes ranging from 0.5 to 6.0 kb. The RAPD profile of the STEC strains showed heterogeneous banding patterns (Fig. 1).

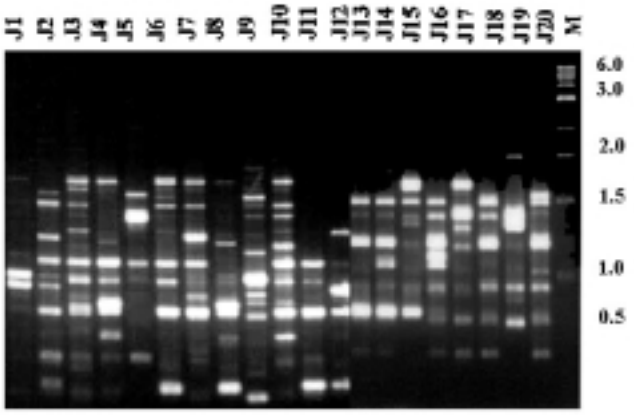

Fig. 1. RAPD-PCR results of stx_1-harbouring *E. coli* isolates. (M, Molecular weight marker (Kb); J1to J20 STEC strains isolated from the clinical diarrhoea patients).

DISCUSSION

To our knowledge, this is the first report on the isolation of *E. coli* strains harbouring *stx* from diarrhoeal patients in Bangladesh. STEC was not associated with diarrhoea in a previous study conducted in this region in 1991-92 [24]. This study revealed a low prevalence of non-O157 *stx1*-harbouring *E. coli* in Bangladesh, and is unlike the reports from Europe, Australia, Brazil and Argentina [25-28] where a much higher incidence of non-O157 STEC was observed. However, the overall incidence of STEC is much lower in developing countries as compared to industrialized countries. The most frequent serotype observed in this study was O44 (20%).

Multidrug resistance was frequent among STEC strains encountered in this study, especially to ampicillin, cephalothin, co-trimoxazole, tetracycline and nalidixic acid. In this study, 45% of the STEC strains were resistant to ciprofloxacin. Due to excessive use of this drug in recent years, many enteric pathogens were reported to be resistant to ciprofloxacin in India [29-32]. Another interesting observation encountered in the present study is the incidence of a higher number of ciprofloxacin-resistant than norfloxacin-resistant STEC strains. The same trend was reported for *V. cholerae* O1 [29] and *Aeromonas* spp. [32]. Even though ciprofloxacin and norfloxacin are basically the same drug, extensive use of the former and a difference in accumulation kinetics between the two are thought to account for the variation in resistance pattern [29].

Analysis of virulence markers indicated that the majority of the non-O157 strains carried *stx1*, whereas the *eae* gene was detected at a very low frequency. Non-O157 STEC strains harbouring *stx1* but lacking *stx2* have been reported earlier [2, 33]. The higher prevalence of *E. coli* with *stx1* genotype was also noted in a study on STEC in the neighbouring region, Kolkata, India [19]. In this study, only two strains harboured the *eae* gene. An increasing number of reports indicate that production of intimin is not essential for the STEC-mediated pathogenesis [18, 34, 35]. One study reported toxin expression by intimin-negative STEC strains (O91:H21 isolates) using only 50% lethal dose in a streptomycin-treated CD-1 mouse model [36]. A subset of human STEC, such as *stx1c*-harbouring strains, generally lacks the intimin-encoding *eae* gene [35, 37]. The factors responsible for colonization by non-O157 strains lacking the intimin are not well established, although many candidate adhesins have recently been reported [14, 38-41]. The STEC strains carrying *eae* are generally considered to have higher virulence for humans than those lacking the *eae* [42]. However, from the available reports [34,43] it is evident that the association between HUS and the absence of *eae* remains unclear.

Recently, Paton and Paton [14] described a novel megaplasmid-encoded adhesin (*saa*) in human STEC lacking the *eae* gene. As most of the *stx1*-harbouring *E. coli* strains were negative for *eae* in this study, we included the *saa* in the investigation for plasmid-associated virulence markers along with other possible additional virulence traits such as STEC haemolysin (*hlyA*), a bifunctional catalse peroxidase (*KatP*), and the *etpD* gene cluster, which encodes a type II secretion pathway [9, 10]. None of the tested isolates produced the specific amplicons.

To confirm the authenticity of *stx* PCR results, we confirmed that the amplicons were specific for *stx1* by Southern hybridisation assay with *stx1*-specific probes prepared from the plasmid pKTN501. The PCR products showed positive signals in the hybridisation assay, confirming that the PCR assay was specific for *stx1*. In a bid to further confirm the presence of *stx1*, we performed sequence analysis of the corresponding *stx1* PCR product and observed 100% homology to the published *stx1* sequences in the database.

A paradoxical observation in this study was that none of the *stx* harbouring strains demonstrated the production of Stx in the Vero cell cytotoxicity assay. A similar observation was made in a study on *stx1* strains with low toxin expression isolated in Thailand [18]. It is a well established fact that the genes encoding the Stx is located in the genomes of temperate bacteriophages [44] and that the phage encodes transcription factor essential to activation of Shiga toxin expression [45]. Recent findings suggest that the *stx* harbouring strains have weak or ineffective phage transcription factor leading to sub-optimal or no production of Stx [46]. It appears that the role of Stx in causing watery diarrhoea is non-essential as evidenced in an earlier study [47].

Our study has, for the first time in Bangladesh, demon-

strated the existence of *stx*-harbouring *E. coli*. The role of *stx1*-harbouring *E. coli* isolates, which lack *eae*, *saa* and other virulence markers, needs to be studied in further detail. The lack of established virulence genes and the non-expression of Stx in our set of *E. coli* strains make it difficult to establish their role in diarrhoea. Our observation of clinical variations in different age groups could not be generalized due to the small sample size. However, the fact that non-O157 STEC are present in the Bangladeshi setting underscore the need to conduct a well-designed study to determine the significance of STEC as a pathogen in this part of the world.

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REAL TIME ESTIMATION OF REPRODUCTION NUMBERS BASED ON CASE NOTIFICATIONS
- Effective reproduction number of primary pneumonic plague

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Abstract: To estimate the time-dependent transmission potential of primary pneumonic plague (PPP), we analyzed historical records from six outbreaks. Based on contact investigation information (source of infection information) of three outbreaks, we generalized the probability density function of the serial interval with a Gamma distribution using maximum likelihood estimations. Furthermore, we used a likelihood-based approach to estimate effective reproduction numbers at time t, R_t, incorporating records from the remaining three outbreaks by assuming independence within unknown contact networks. According to our estimates, the R_t of PPP during the initial phases of each epidemic were roughly in the order of 1.3 (95% confidence interval (CI): 0.0-4.3) in Oakland, 1.4 (0.0-4.6) in Rangoon and 6.5 (0.0-16.0) in Ecuador. The expected values of R_t were shown to slightly exceed unity, even in latter stages. While declining trends in R_t were observed in Oakland and Ecuador, no such trend was observed in Rangoon. The findings suggest that the three outbreaks investigated could have been accompanied by demographic stochasticity. The statistical usefulness of the transformation procedure, even with a small number of recorded cases available, was demonstrated, and the expected responses to bioterrorism using *Yersinia pestis* were discussed.
Key words: Primary pneumonic plague; *Yersinia pestis*; Reproduction number; Maximum likelihood estimation

INTRODUCTION

Plague is a zoonosis whose etiological agent is *Yersinia pestis*, a gram-negative bacillus. This bacterium is believed to have been the cause of three past pandemics in the 6th, 14th, and 20th centuries (McGovern & Friedlander, 1997). Despite the recent decline in annual incidence (World Health Organization, 2003), plague outbreaks as a result of biological warfare remain a possibility (Inglesby et al., 2000). Intentional dissemination of plague would most probably implement an aerosolized form of *Y. pestis*, which has been shown to produce the disease in nonhuman primates and to result in symptoms initially resembling those of other severe respiratory illnesses (Speck & Wolochow, 1957). A plague epidemic following the use of *Y. pestis* as a biological weapon would be caused by airborne transmission, leading to person-to-person transmission via respiratory droplets.

Primary pneumonic plague (PPP) is contracted naturally by inhalation of airborne bacilli from cadavers or carcasses of animals, or more commonly through droplet infection from coughing (Pollitzer, 1954). Case fatality could reach almost 100% without appropriate antibiotic treatment within 24 hr after onset of symptoms (Wu, 1926). It is said that Soviet scientists manufactured large quantities of *Y. pestis* during the Cold War, allegedly engineering multidrug -resistant strains (Inglesby et al., 2000). These resistant strains have also been found naturally (Galimand et al., 1997). The World Health Organization (WHO) stated that, in a worst-case scenario, 50kg of *Y. pestis* released as an aerosol over a city of five million could result in 150,000 cases of PPP, with 80,000--100,000 requiring hospitalization and 36,000 resulting in death (World Health Organization, 1970).

Since human susceptibility to plague is thought to be universal and since the efficacies of existing vaccines have not been proven (Titball & Williamson, 2001), the introduction of aerosolized plague into the general population could

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constitute a significant public health problem because of the transmissibility of this bacterium. The greater the transmissibility of the bacteria the greater the potential for spread, thus the more intense and rapid the intervention strategies must be to bring it under control. Although reasonable estimates of the basic reproduction number, R_0, of a pneumonic plague, defined as the average number of secondary cases per primary case, have been obtained on the basis of historical analyses (Gani & Leach, 2004), we still do not know how past epidemics were terminated and how the reproduction number varies with time. That is, values of R_0 alone do not allow a thorough interpretation and investigation of how we should be preparing for potential future bioterrorist attacks. On the other hand, estimates of the effective reproduction number (R_t), defined as the reproduction number at time t with or without introduction of control measures and depletion of susceptible individuals, allow elucidation of the future course of an outbreak; typically, $R_t < R_0$. The purpose of this study was to provide a rational assessment of the transmissibility of PPP within a statistical framework by referring to historical records.

MATERIALS AND METHODS

2.1. Subjects

To estimate transmissibility with time, represented by the effective reproduction number (R_t; see Section 2.3), we utilized historical records from outbreaks caused exclusively by PPP in the last century. Case records of PPP outbreaks caused by *Y. pestis* are limited, since most references describe not only PPP but also bubonic, septicemic, and secondary pneumonic plague outbreaks. In addition, the source of inhalation of the bacillus can arise, not only from humans, but also from animals such as dogs, cats, and pigs (Doll et al., 1994). To estimate the reproduction number of PPP, we confined our investigation to reports that met the following conditions: (i) must state that the outbreak was caused by PPP only, confirmed clearly through epidemiologic, clinicopathological or bacteriological examinations, (ii) the location of the outbreak must not have experienced bubonic plague within a relatively similar time frame, thereby excluding the possibility of unrelated deaths and transmission by bubonic, septicemic and secondary pneumonic plague, and (iii) contact patterns in at least some of the outbreaks must have been traced, giving the statistical distribution of the serial interval (= generation interval; Wallinga & Teunis, 2004), defined as the mean of the infectiousness distribution and representing the time from symptom onset in a primary case to symptom onset in a secondary case (Fraser et al., 2004). Historical records from a total of six outbreaks were examined. Three records included complete or partly complete information on the sources of infection; these were related to outbreaks in Mukden (China, 1946; Tieh et al., 1948), Northwestern Madagascar (1957; Brygoo & Gonon, 1958), and, more recently, Central Madagascar (1997; Ratsitorahina et al., 2000), and were analyzed to extrapolate the statistical distribution of the serial interval. The remaining three records were obtained from outbreaks in Oakland (the United States, 1919; Kellogg, 1920), Ecuador (1939; Murdock, 1940) and Rangoon (Burma, 1946; Wynne-Griffith, 1948) but were devoid of information regarding who infected whom; these were used to estimate the reproduction number. In all outbreaks, the index case was infected a certain distance from the location of the outbreak before the disease was introduced into the affected community.

2.2. Estimations of the serial interval

Since information concerning background demography and the number of infectious contacts could not be obtained, and since the number of patients in each record was small, we used are a likelihood-based approach to estimate the reproduction number presented in this paper. We obtained the serial interval through evaluations of the course of the outbreaks (investigated chains of transmission) in Mukden and NW and Central Madagascar using information on the time of onset of each recorded infected individual. In all three outbreaks, contact patterns were clearly traced (Tieh et al., 1948; Brygoo & Gonon, 1958; Ratsitorahina et al., 2000). The final size of each outbreak, denoted by 'n' throughout this paper, was 39 cases in Mukden, 41 in NW Madagascar, and 18 in Central Madagascar. Of the total 98 cases, 89 intervals from onset of primary to onset of secondary cases were obtained, excluding cases without an associated link with another case. Since the total number of recorded cases in each outbreak was too small for separate estimations of the statistical distributions of each serial interval, an overall estimate was obtained by summing the three settings.

We assumed that the serial interval, τ, follows typical standard distributions: Gamma, Weibull and log-normal distributions. The probability density function (pdf) of the gamma distribution denoted by $f(\tau \mid \alpha, \beta)$ is given by:

$$f(\tau;\alpha,\beta) = \frac{\beta^{\alpha}}{\Gamma(\alpha)}\tau^{\alpha-1}\exp(-\beta\tau)(\tau \geq 0) \qquad (1)$$

where Γ(a) is the Gamma function represented by:

$$\Gamma(\alpha) = \int_0^{\infty} x^{\alpha-1}e^{-x}dx \qquad (2)$$

Here, α and $1/\beta$ represent shape and scale parameters, respectively, and are restricted to being positive real. On the other hand, pdf of Weibull and log-normal distributions, $\omega(\tau \mid \gamma, \delta)$ and $l(\tau \mid \mu, \sigma^2)$, are given by

$$\omega(\tau;\gamma,\delta) = \delta\gamma\tau^{\gamma-1}\exp(-\delta\tau^{\gamma}) \quad (3)$$

and

$$l(\tau;\mu,\sigma^2) = \frac{1}{\sqrt{2\pi}\sigma\tau}\exp\left\{-\frac{(\ln\tau-\mu)^2}{2\sigma^2}\right\} \quad (4)$$

where γ and δ are called shape and scale parameter, respectively, while μ and σ^2 are mean and variance. Numbers of cases in each outbreak were stratified according to the serial interval [days]. Parameter estimates were obtained by minimizing the negative logarithm of the likelihood function using a standard numerical algorithm (maximum likelihood estimation). Finally, quantile plot was used to determine the best fits of distribution: the goodness-of-fit was assessed visually since the standard goodness-of-fit test statistic for the standard distribution without fixing parameters is unavailable. For instance, a quantile plot, with the selected gamma quantiles on the vertical axis and variable values on the horizontal axis, was drawn to confirm the validity of the selected model (gamma distribution).

2.3. Estimations of reproduction number

Using the obtained distribution of the serial interval and the remaining three outbreak records, which included information on the date of onset but were devoid of data regarding who infected whom, estimations of R_t at time t were performed. The usefulness of a recently proposed statistical method allowing reasonable real-time transformation of epidemic curves with the reproduction number over time was investigated (Wallinga & Teunis, 2004). This method theoretically assumes all probable transmission networks of who infected whom from pairs of cases. Here, for instance, using the serial interval with a gamma distribution, the time between onset of cases '*i*' and '*j*' is denoted by $t_i - t_j$, and the probability of transmission is given by $f(t_i - t_j)$. It is assumed that q cases out of the total number of cases, n, were infected outside the community investigated. The vector ν denotes the structure of the transmission network while $\nu(i)$ represents the i th element of ν in which the primary case infected case '*i*'. Consequently, the likelihood of observing epidemic curve t, with θ (population parameter) representing the serial interval and ν for the network, is:

$$L(\mathbf{v},\theta \mid \mathbf{t}) = \prod_{i=1}^{i=n-q} f(t_i - t_{\nu(i)} \mid \theta). \quad (5)$$

Further details of this concept are given in Wallinga and Teunis (2004). The relative likelihood that case '*l*' has been infected by case '*m*' is consequently given by:

$$p_{(l,m)} = \frac{L(V_{(l,m)},\theta \mid \mathbf{t})}{L(V,\theta \mid \mathbf{t})} = \frac{f(t_l - t_m \mid \theta)}{\sum_{s=1,s\neq l}^{s=n} f(t_l - t_s \mid \theta)} \quad (6)$$

and finally, the reproduction number is given by:

$$R_t = \sum_{k=1}^{k=n-q} Bi[p_{(k,l)}] \quad (7)$$

where $Bi[\ ]$ denotes the binomial distribution. Estimates are thus obtained without any unrealistic assumptions, except that all probable contacts are taken into account by assuming the independence.

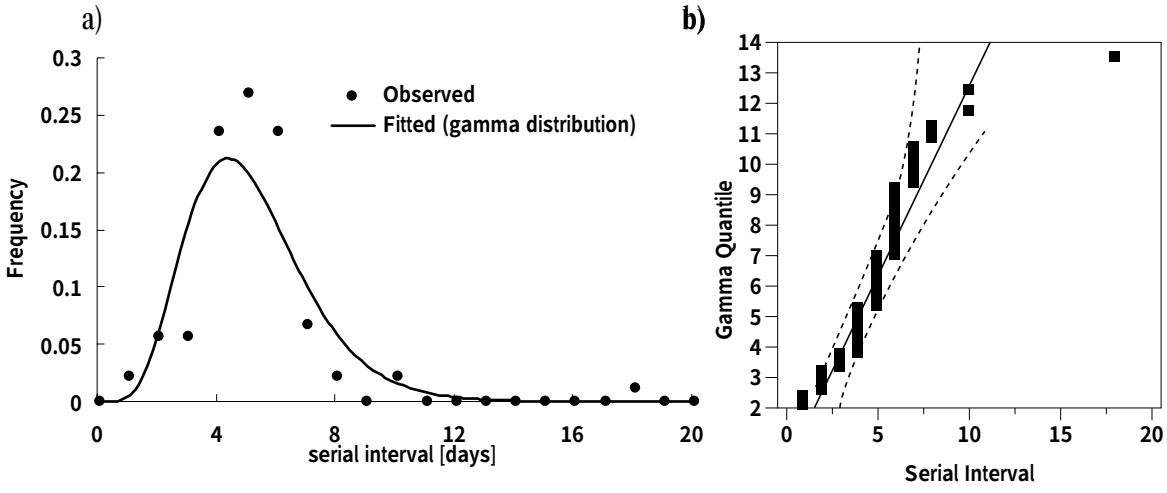

Figure 1. a) Estimated serial intervals of known transmissions of primary pneumonic plague in Mukden (1946), NW Madagascar (1957) and Madagascar (1997). The serial interval is the time from onset of symptoms in an index case to onset of symptoms in a secondary case (also called the generation time / interval). The vertical axis shows the frequency (probability density). **b)** The quantile plot with gamma quantiles on the vertical axis. The diagonal reference line shows the line of fit while the two dashed lines denote confidence limits of 95% equal precision bounds with a= 0.001 and b=0.99 (Meeker and Escobar, 1998)).

RESULTS

3.1. The serial interval

The median serial interval of the three outbreaks was 5.0 days with a standard deviation (SD) of 2.1 (Fig. 1a). Although the serial interval at initial stages of an outbreak tends to be longer than that at latter stages (Lipsitch et al., 2003), we ignored this tendency due to the scarcity of recorded subjects. Maximum likelihood estimates of the shape and scale parameters of the gamma distribution were 6.3 and 0.8, respectively, and the skewness and kurtosis were 2.3 and 14.3, respectively. Weibull and log-normal distributions were not selected as the best fit since the quantile plots were more typically 'snake-shaped' than those of gamma distribution. The gamma distribution with these parameters appeared to adequately describe the data; the majority of the data points and the quantiles for the hypothesized gamma distribution were relatively close (Fig. 1b).

3.2. Reproduction number

Comparative representations of epidemic curves and estimated reproduction numbers of each outbreak are shown in Fig. 2. The final sizes of each outbreak, '*n*', were 13 cases in Oakland, 13 in Rangoon and 18 in Ecuador. Each was terminated almost 1 month after introduction of an index case. The observed patterns of spread did not show particularly propagated (person-to-person) epidemic curves; rather they qualitatively showed intermittent-like patterns (Dwyer & Groves, 2003). The effective reproduction numbers, R_t, of PPP at the initial phase of each epidemic were roughly in the order of 1.3 (95% confidence interval (CI): 0.0-4.3) in Oakland, 1.4 (0.0-4.6) in Rangoon and 6.5 (0.0-16.0) in Ecuador. In these three outbreaks, the expected values of R_t slightly exceeded unity even in latter stages. Due to the scarcity of recorded cases, the 95% CI were rather wide. A qualitatively declining trend in R_t was seen in Oakland and Ecuador but not in Rangoon. In the former two outbreaks, the upper 95% CI also decreased, but not below unity in latter stages.

DISCUSSION

The outbreaks studied here met our original requirements of being caused by PPP only. Despite problems with the limited total number of cases in selected records, the simple statistical methods gave reasonable estimations of both the serial interval and reproduction number. For the purpose of these estimations, we used the probability density function of the serial interval obtained from three different outbreaks, giving a median value of 5.0 days. Even though demographic information was lacking, the overall range of expected R_t values in initial stages was determined

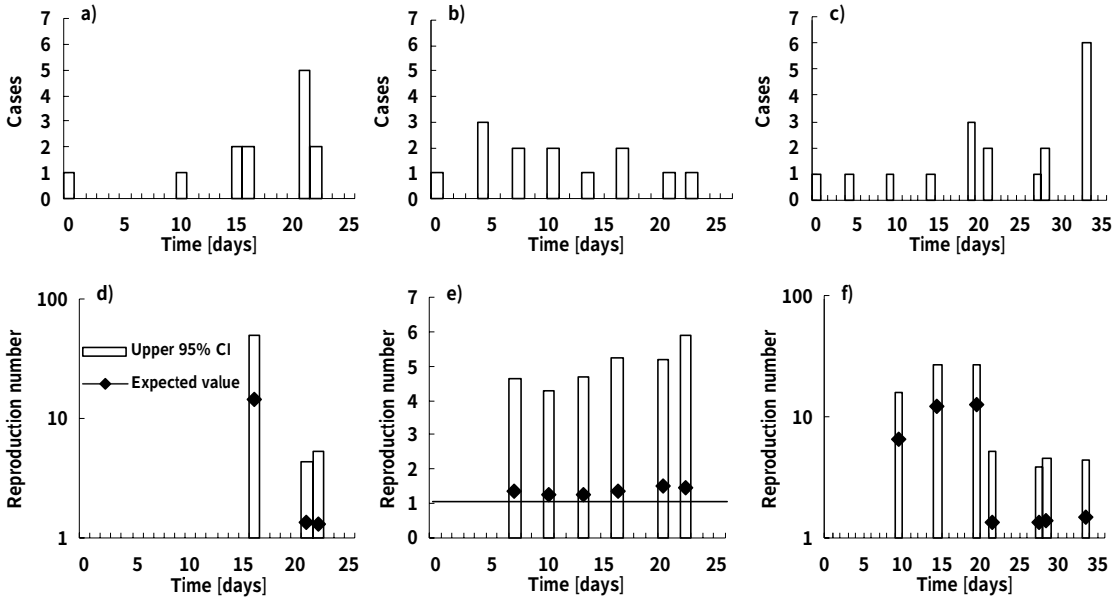

Figure 2. Epidemic curves (numbers of cases according to the date of onset) for primary pneumonic plague outbreaks in a) Oakland (1919), b) Rangoon (1946), and c) Ecuador (1939), and corresponding effective reproduction numbers (R_t) (numbers of secondary infections generated per primary case according to the date of onset) in d) Oakland, e) Rangoon, and f) Ecuador. Dots show expected values while the top of the bar charts denotes the upper 95% confidence interval (CI). The horizontal axis represents the lower 95% CI. The solid horizontal line in (e) indicates R_t=1, and the vertical axis in (d) and (f) is logarithmic.

as being in the order of 1.3-6.5. On the other hand, crude and wide 95% CI of R_t was obtained on the basis of the assumption of binomial distributions for the relative likelihood.

Although it might not be possible to demonstrate the exact estimates of R_t with better precision, the estimates and range of R_t obtained provide important insights. Our estimations revealed that the expected values of R_t in the three analyzed outbreaks did not decline below unity even when very close to extinction. Actually, in most regions, except Mukden (the records of which were used only for estimating serial interval) where interventions were carried out, outbreaks came to an end before control measures were implemented. Thus, the findings suggest that the outbreaks investigated could have been accompanied by certain demographic stochasticity (Iwasa & Mochizuki, 1988). However, the impact of extrinsic factors (i.e., public health intervention) on the trends remained unclear.

A study of notorious outbreaks in China (Pollitzer & Li, 1943) suggested the reason for the end of the Mukden outbreak; that is, PPP is generally not as infectious as measles or influenza. It has also been frequently suggested, even before the mid-20th century, that transmission of PPP resulted in many cases from close or actual contact with an infected person (Meyer, 1961). Among our subjects, however, depletion of the number of susceptibles due to the rural nature of the outbreak locations is also highly plausible, and this factor, together with stochastic effects, could have brought these outbreaks to an end. This may also help to explain why outbreaks tended to continue even after control measures were introduced in highly populated areas in China (International Plague Conference, 1912). These complicated circumstances have made it difficult to interpret previous outbreaks in detail, and the transmission potential of PPP has thus remained largely unknown (Wright, 1995). Even though it is not possible to draw definite conclusions from our results, we suggest that some outbreaks end as a result of stochastic effects as well as intrinsic factors (depletion of susceptibles), especially in rural areas where most outbreaks occur.

The advantages and limitations of the estimation method used should be addressed. This study demonstrated the statistical usefulness of the real-time transformation procedure, even in settings where the number of cases is limited. Previous methodologies for estimation of reproduction number have frequently assumed simple exponential growth without proper statistical validation, generating statistically flawed results (Donnelly et al., 2004). Assessments within an autonomous system, which lack time-dependent information, have therefore not been helpful in drawing quantitative conclusions with real life data (Diekmann & Heesterbeek, 2000). The dangers of intuitively fitting exponential growth curves to cumulative case numbers have already been argued (Razum et al., 2003), and such technical flaws, due to assumption of constant exponential growth despite a significantly different qualitative pattern, were our primary concern in this study. Despite considerations of the individual variability of transmission potential and of stochasticity in the simulations, recent literature documenting estimations of the R_0 of PPP have been accompanied by the above flaw (Gani & Leach, 2004). Our study was improved by showing variation in the reproduction number with time. However, as urged with analyses of SARS, it should be noted that conditions of independence, as described in the methods section, and variations in the serial interval with time could be limiting factors (Lipsitch & Bergstrom, 2004). In addition, it is imperative that the disease be categorized by combining the records of different outbreaks. Since the strain of *Y. pestis* varies with time and place, not only the degree of infectivity of the bacilli but also the serial interval (approximated by the sum of incubation and symptomatic periods) will differ. Because of biological and socio-environmental differences, it is obvious that local characteristics of transmission can vary largely and may show overly specific originalities. Insightful investigations on this point are a subject for our furher study. Finally, although reconstructed epidemic trees enable us to estimate the R_t directly, this is possible only when “who infected whom” is observed completely or parly known. We intend to discuss this issue in a later paper (Nishiura, Schwehm, Kakehashi & Eichner, Submitted).

It is believed that the Japanese army attempted to drop plague-infected fleas over populated areas as a biological weapon during World War II (Harris, 1994). Taking into account its availability and stability compared to other viral agents, PPP is one of the most likely threats in a bioterrorist attack. Though extinction occurred in all outbreaks observed here, a large-scale initial attack in a highly populated area will not offer demographic stochasticity. Considering the extremely high case fatality that occurs without rapid treatment (Wu, 1926) and the unavailability of immunization (Titball & Williamson, 2001), preparation of a public health response is of outstanding imporance. To overcome the fear of bioterrorism using *Y. pestis*, both statistical and mathematical studies should be implemented to promote the development of a response program.

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TWO NEW SPECIES OF *SIMULIUM* (*NEVERMANNIA*) (DIPTERA: SIMULIIDAE) FROM NORTHERN THAILAND

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Abstract: Two new species of black flies, *Simulium* (*Nevermannia*) *fruticosum* sp. nov. and *S*. (*N*.) *chiangklangense* sp. nov. are described on the basis of samples collected in northern Thailand. These new species are assigned to the *feuerborni* species-group of the subgenus *Simulium* (*Nevermannia*), and are easily distinguished from *S*. (*N*.) *feuerborni* Edwards, the only species of the same group known thus far in Thailand, by their simple cocoon without any anterodorsal projection.
Key words: *Simulium*, black fly, Simuliidae, Thailand, new species, *Nevermannia*.

In Thailand, the *feuerborni* species-group within the subgenus *Simulium* (*Nevermannia*) Enderlein redefined by Takaoka [1] is represented by only one species, *S*. (*N*.) *feuerborni* Edwards, which was originally described from Java [2] and later recorded from Peninsular Malaysia [3], Thailand [4] and Sumatra [5]. We collected two more species of the *feuerborni* species-group, which are easily distinguished from *S*. (*N*.) *feuerborni* by their simple cocoon. These are described here as new species.

The terms for morphological features used here follow those of Takaoka [1]. Holotype and paratype specimens of the new species are deposited at the Department of Infectious Disease Control, Oita University, Oita, Japan.

Simulium (*Nevermannia*) *fruticosum* sp. nov.

DESCRIPTION. **Female.** Body length 2.4−3.0 mm. ***Head.*** Narrower than thorax. Frons brownish-black, not shiny, thinly whitish-grey pruinose, densely covered with whitish-yellow recumbent hairs, interspersed with several dark longer and stouter hairs along each lateral margin. Frontal ratio 1.3−1.7 : 1.0 : 2.0−2.5. Frons-head ratio 1.0 : 4.8−5.2. Fronto-ocular area (Fig. 1A) well developed, triangular, directed somewhat upward. Clypeus brownish-black, not shiny, whitish-grey pruinose, densely covered with whitish-yellow recumbent hairs (except portion near upper margin bare), intermixed with several dark longer and stouter hairs on each side of lower 1/2. Labrum nearly as long as clypeus. Antenna composed of 2+9 segments, medium to dark brown, except scape, pedicel, and minute base of 1st flagellar segment yellow or light yellowish-brown; 1st flagellar segment 2.0−2.2 times as long as 2nd one. Maxillary palp consisting of 5 segments, medium to dark brown, proportional lengths of 3rd, 4th, and 5th segments 1.0 : 0.7−0.8 : 1.3−1.5; 3rd segment (Fig. 1B) much enlarged, with sensory vesicle elongate, 0.58−0.66 times as long as 3rd segment, with medium-sized opening medially. Maxillary lacinia with 8 or 9 inner teeth and 12−14 outer ones. Mandible with 20−22 inner teeth but lacking outer teeth. Cibarium (Fig. 1 C) smooth on posterior margin, with well sclerotized arms directed anterolaterally. ***Thorax.*** Scutum light to dark brown (except anterolateral calli ocherous) though somewhat darker along lateral margins and on prescutellar area, shiny at certain angles of light, thinly whitish-grey pruinose, with 3 faint dark narrow longitudinal vittae (1 medial and 2 submedial), densely covered with whitish-yellow (golden in light) recumbent hairs, and with several dark brown upright hairs on prescutellar area. Scutellum ocherous, with many dark upright hairs as well as whitish-yellow shorter hairs. Postnotum medium to dark brown, thinly whitish-grey pruinose, slightly shiny at certain angles of light, bare. Pleural membrane bare. Katepisternum longer than deep, medium brown, and bare. ***Legs.*** Foreleg: coxa and trochanter whitish-yellow; femur yellow to dark yellow with apical cap medium brown; tibia medium to dark brown with median large portion of outer surface light brown; tarsus brownish-black; basitarsus slightly dilated, 7.7 times as long as its greatest width. Midleg: coxa light brown with posterior surface dark brown; trochanter yellow; femur yellow to dark yellow, with apical cap medium brown; tibia

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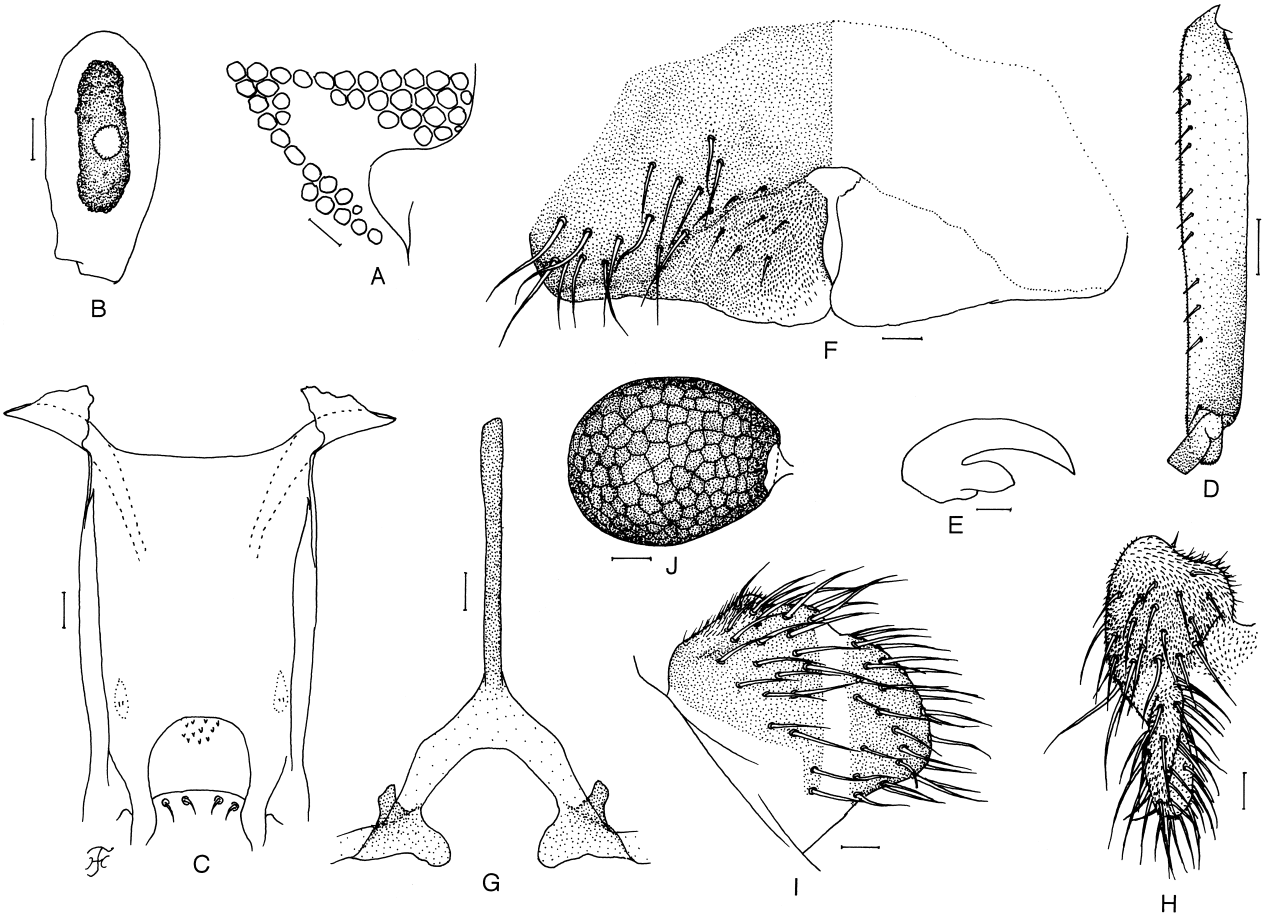

Fig. 1. Female of *Simulium* (*Nevermannia*) *fruticosum* sp. nov. A, fronto-ocular area (right side); B, 3rd segment of maxillary palp with sensory vesicle (left side, front view); C, cibarium; D, basitarsus and second tarsal segment of hind leg showing calcipala and pedisulcus (left side, outer view); E, claw; F, 8th sternite and ovipositor valves *in situ* (ventral view); G, genital fork (ventral view); H and I, paraprocts and cerci (right side; H, ventral view; I, outer view); J, spermatheca. Scale bars. 0.1 mm for D; 0.03 mm for A and B; 0.02 mm for F–J; 0.01 mm for E.

medium brown with apical cap dark brown and median large portion on outer and inner surfaces light brown; tarsus brownish-black. Hind leg: coxa dark yellow or light brown; trochanter yellow; femur yellow to dark yellow with apical cap medium to dark brown; tibia medium brown with apical cap dark brown and median large portion on outer and inner surfaces light brown; tarsus medium to dark brown except basal 1/2 (or a little more) of basitarsus and basal 1/2 of 2nd segment dark yellow to light brown (though base of basitarsus medium brown); basitarsus (Fig. 1D) nearly parallel-sided on basal 1/2, then slightly narrowed toward apex, 6.1 times as long as wide, and 0.77 times and 0.63 times as wide as hind tibia and femur, respectively; calcipala (Fig. 1 D) well developed, about 1.1 times as long as wide, and about half as wide as greatest width of basitarsus; pedisulcus (Fig. 1D) well developed. Claws (Fig. 1E) each with large basal tooth half as long as claw. ***Wing.*** Length 2.4–2.6 mm. Costa with 2 parallel rows of dark short spines as well as dark hairs except subbasal portion of costa near humeral cross vein with whitish-yellow hairs. Subcosta with dark hairs except near apex bare. Basal portion of radius fully haired; R_1 with dark spinules and hairs; R_2 with dark hairs. Hair tuft on stem vein dark brown. Basal cell absent. ***Abdomen.*** Basal scale light to medium brown, with fringe of pale long hairs. Dorsal surface of abdomen medium to dark brown except that of segment 2 whitish-yellow or ocherous to light brown (though tergal plate light to medium brown), moderately covered with whitish-yellow short hairs, interspersed with dark ones; tergite 5 slightly shiny at certain angle of light, tergites 6–9 shiny when illuminated; ventral surfaces of abdominal segments 2–6 whitish-yellow; segment 7 with large sternal plate medially. ***Genitalia.*** Sternite 8 (Fig. 1F) wide, bare medially but furnished with 18–43 short and long hairs on each side. Ovipositor valves

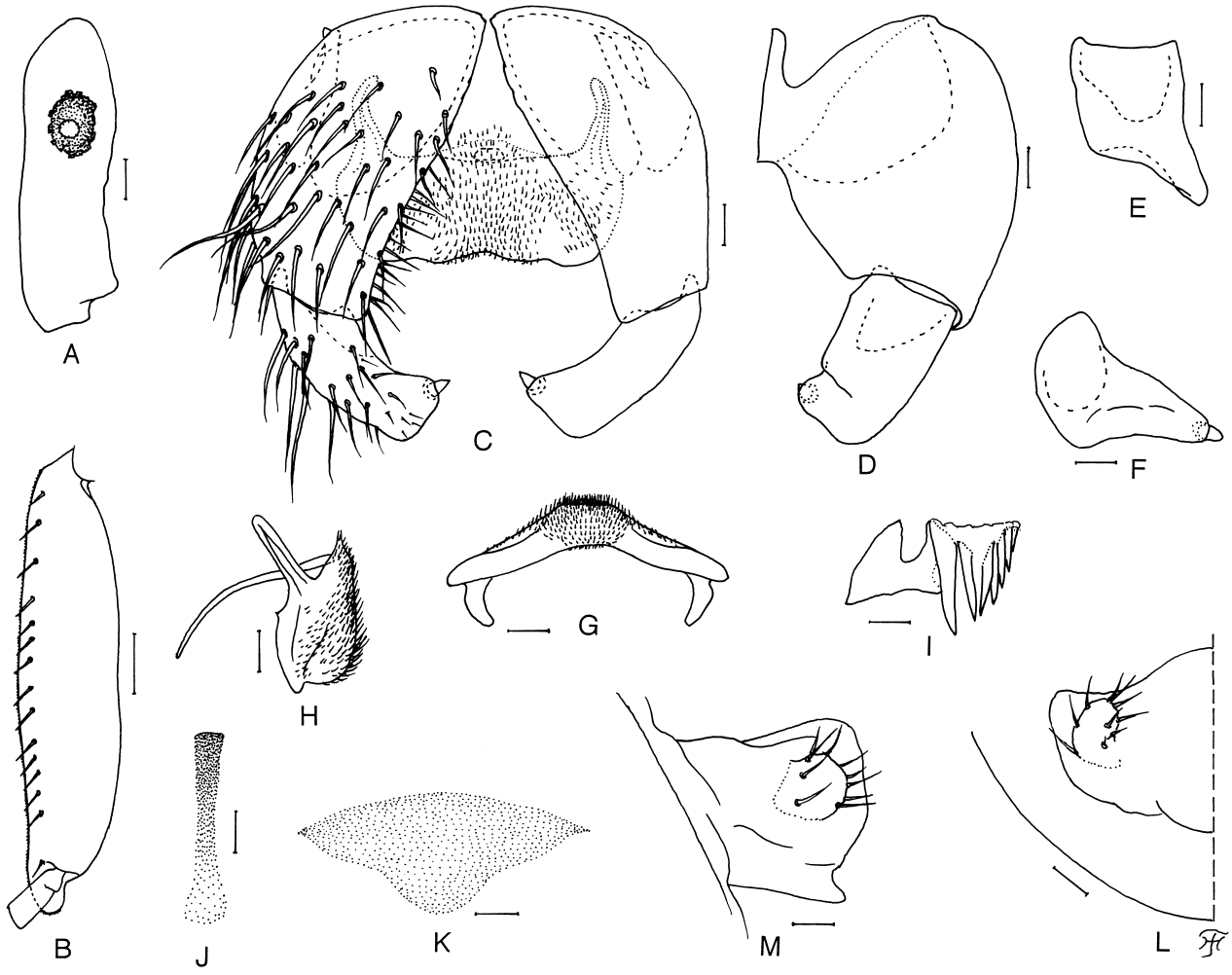

Fig. 2. Male of *Simulium* (*Nevermannia*) *fruticosum* sp. nov. A, 3rd segment of maxillary palp with sensory vesicle (right side, front view); B, hind basitarsus (left side, outer view); C, coxites, styles and ventral plate *in situ* (ventral view); D, coxite and style (right side; outer view); E and F, styles (right side; E, ventrolateral view; F, end view); G, ventral plate (end view); H, ventral plate and median sclerite (lateral view); I, paramere (right side; end view); J, median sclerite; K, dorsal plate; L and M, 10th abdominal segments with cercus (right side; L, end view; M, outer view). Scale bars. 0.1 mm for B; 0.02 mm for A, C–M.

(Fig. 1F) triangular, thin, membraneous except inner margin narrowly sclerotized, densely covered with microsetae, interspersed with 5–7 short setae; inner margins gently sinuous and narrowly separated medially from each other. Genital fork (Fig. 1G) of inverted Y-form, with well sclerotized stem and wide arms; each arm with wide round lobe-like projection directed medioposteriorly and prominent projection directed forward. Paraproct (Fig. 1H, I) of usual form, only slightly protruding ventrally, with 24–30 hairs on ventral and lateral surfaces, and with 5–8 sensilla on anteroinner surface. Cercus in lateral view (Fig. 1I) rounded posteriorly, 0.5–0.7 times as long as paraproct. Spermatheca (Fig. 1J) ovoidal, about 1.2 times as long as its greatest width, strongly sclerotized except small area around juncture to duct, and duct itself unsclerotized, with distinct reticulate surface pattern, and without internal setae; main spermathecal duct narrow, while both accessory ducts slightly wider than main duct.

Male. Body length 2.6–3.2 mm. ***Head.*** Slightly wider than thorax. Holoptic; upper eye consisting of large facets in 14 or 15 vertical columns and 17 or 18 horizontal rows. Clypeus dark brown, not shiny, whitish-grey pruinose, moderately covered with yellow short and long hairs interspersed with dark long hairs. Antenna composed of 2+9 segments, light brown except base of 1st flagellar segment pale yellow, or dark brown except scape and most of pedicel

dark yellow and base of 1st flagellar segment pale yellow; 1st flagellar segment elongate, about twice as long as 2nd one. Maxillary palp medium to dark brown, composed of 5 segments, proportional lengths of 3rd, 4th, and 5th segments 1.0:1.0−1.2:1.7−2.1; 3rd segment (Fig. 2A) of moderate size; sensory vesicle (Fig. 2A) small, globular or ellipsoidal, 0.22 times as long as 3rd segment. ***Thorax.*** Nearly as in female except anterolateral calli light brown and postnotum light to dark brown. ***Legs.*** Foreleg: coxa and trochanter yellow; femur light brown with apical cap medium brown (though most of inner surface lighter); tibia dark brown to brownish-black with median large portion of outer surface medium brown; tarsus brownish-black; basitarsus very slightly dilated, 10 times as long as its greatest width. Midleg: coxa medium brown with posterior surface dark brown; trochanter yellow; femur yellow to dark yellow, with apical cap medium brown; tibia dark brown to brownish-black with base and subbasal portion medium brown; tarsus brownish-black. Hind leg: coxa light brown; trochanter yellow; femur yellow to dark yellow with apical cap dark brown; tibia dark brown to brownish-black with base dark yellow and subbasal portion light to medium brown; tarsus dark brown except basal 1/2 of basitarsus medium brown though border not well defined; basitarsus (Fig. 2B) enlarged, spindle-shaped, 4.2 times as long as its greatest width, and 1.04 times and 0.96 times as wide as greatest widths of hind tibia and femur, respectively; calcipala (Fig. 2B) well developed, nearly as long as wide, and 0.35 times as wide as greatest width of basitarsus; pedisulcus (Fig. 2B) well developed. ***Wing.*** As in female except subcosta bare or with 1 or 2 hairs; length 2.5−2.6 mm. ***Abdomen.*** Basal scale dark brown, with fringe of yellow long hairs. Dorsal surfaces of abdominal segments medium to dark brown (except that of segment 2 pale yellow though tergal plate medium to dark brown), not shiny, covered with dark simple hairs; ventral surfaces of abdominal segments 2−4 yellow though sternites of segments 3 and 4 medium brown; ventral surfaces of segments 5−8 light to medium brown. ***Genitalia.*** Coxite in ventral view (Fig. 2C) subquadrate much longer than wide. Style in ventral view (Fig. 2C) short, 0.7 times as long as coxite, bent inwardly, nearly parallel-sided, with stout spine apically; style in ventrolateral view (Fig. 2 E) broad, nearly parallel-sided from base to a little beyond middle, then abruptly tapered apically; style in end view (Fig. 2F) tapered inwardly. Ventral plate in ventral view (Fig. 2C) lamellate, much shorter than wide, well sclerotized, with 3 concavities on posterior margin, and moderately covered with fine short setae on ventral surface (except lateral portions partially setose); arm of moderate length, slender, and curved inwardly; ventral plate in end view (Fig. 2G) with fine short setae centrally on posterior surface; ventral plate in lateral view (Fig. 2H) with ventral margin of body nearly straight, and arm directed dorsally and forwardly. Paramere (Fig. 2I) with 6 or 7 hooks of different sizes. Median sclerite in end view (Fig. 2J) simple, club-shaped, narrow, slightly widened toward apex. Aedeagal membrane moderately covered with spinous microsetae, moderately sclerotized basally forming dorsal plate (Fig. 2 K). Ventral surface of 10th segment without any hairs near each posterolateral corner. Cercus (Fig. 2L,M) small, rounded and with 9−11 simple hairs.

Pupa. Body length 3.0−3.5 mm. ***Head.*** Integument dark yellow to light brown, densely covered with round tubercles; antennal sheaths bare; frons with 2 short slender simple trichomes (Fig. 3A) on each side; face with1long simple trichome (Fig. 3B) (a little over 3 times as long as frontal trichomes) on each side. ***Thorax.*** Integument dark yellow to light brown, moderately or densely covered with round tubercles, with 3 very long slender simple trichomes with coiled apex (Fig. 3C) mediodorsally, 2 slender simple trichomes (1 very long, and 1 short) (Fig. 3D) mediolaterally, 1 long slender simple trichome (Fig. 3E) posterolaterally, and 3 short slender simple trichomes (Fig. 3F) ventrolaterally, on each side. Gill with 6 long thread-like slender filaments arranged in 2 groups (1 ventral group consisting of paired filaments with long stalk, and the other dorsal group consisting of 4 filaments), arising from short basal common stalk (Fig. 3G); 4 filaments of dorsal group usually arising close together, with their arrangement somewhat variable, e.g., lying nearly side by side horizontally forming middle pair with very short stalk and inner and outer individual filaments (Fig. 3H), or forming outer triplet plus1inner individual filament (Fig. 3I), or forming inner pair with no stalk and outer pair with very short stalk (Fig. 3J), or forming inner and outer pairs with very short to short stalk (Fig. 3K) (as an exception, in 1 pupa stalk of inner pair of dorsal group long on left side and very long on right side as shown in Fig. 3L,M); basal common stalk with transparent bulb-like organ ventrally (Fig. 3G); all filaments dark brown, directed forward, tapered apically, subequal in length to one another (lengths from base of gill to tips of filaments variable from 3.8 to 4.8 mm depending upon individual pupae), and longer than pupal body; cuticular surface with distinct annular ridges and furrows (though ridges becoming indistinct apically), and densely covered with minute tubercles of different sizes (larger ones on ridges and smaller ones on interridges). ***Abdomen.*** Dorsal surfaces of all segments weakly sclerotized, yellowish except medial portions of segments 1 and 2 light brown, densely and elaborately covered with minute tubercles; segment 1 with 1 medium-long slender simple seta on each side; segment 2 with 1 medium-long slender simple seta and 5 short spinous

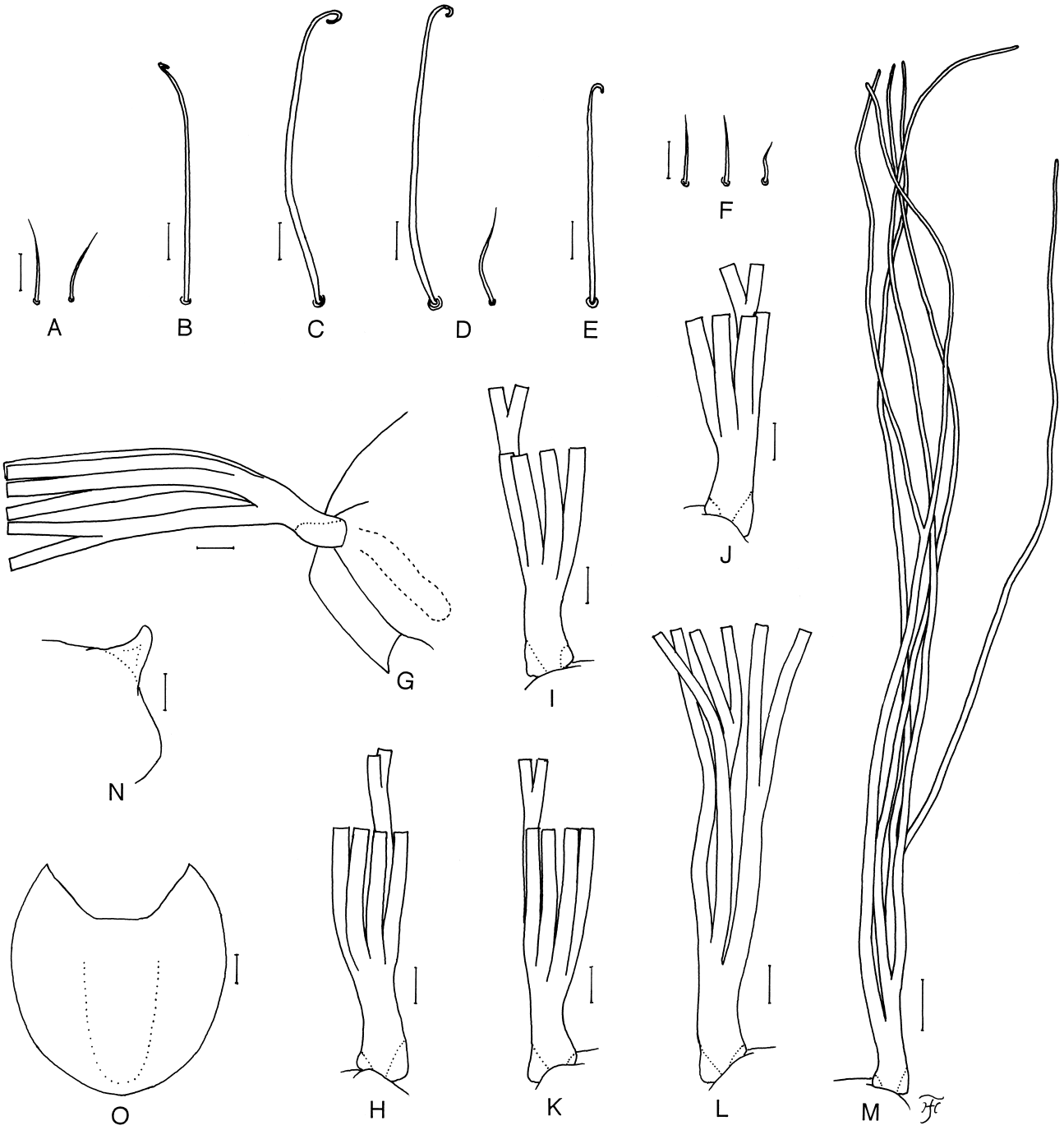

Fig. 3. Pupa of *Simulium* (*Nevermannia*) *fruticosum* sp. nov. A, frontal trichomes; B, facial trichome; C–F, trichomes on thorax (C, mediodorsal; D, mediolateral; E, posterolateral; F, ventrolateral); G, basal portion of gill filaments (left side, outer view); H–L, basal portions of gill filaments (all dorsal view; H and J, left side; I, K and L, right side); M, entire gill filaments showing very long stalk of inner pair of dorsal group (dorsal view); N, terminal hook (lateral view); O, cocoon (dorsal view). Scale bars. 0.5 mm for O; 0.2 mm for M; 0.1 mm for G–L; 0.02 mm for A–F and N.

setae on each side; segments 3 and 4 each with 4 hooks and 1 short spinous seta on each side; segment 5 bare or with 1 or 2 spine-combs on each side; segments 6–8 each with spine-combs directed backward in transverse row on each side; segments 6–9 each with comb-like groups of minute spines on each side; segment 9 with a pair of distinct cone-shaped terminal hooks curved posteroinwardly (Fig. 3N). Lateral surfaces of segments 2–4 each with 3 spinous setae

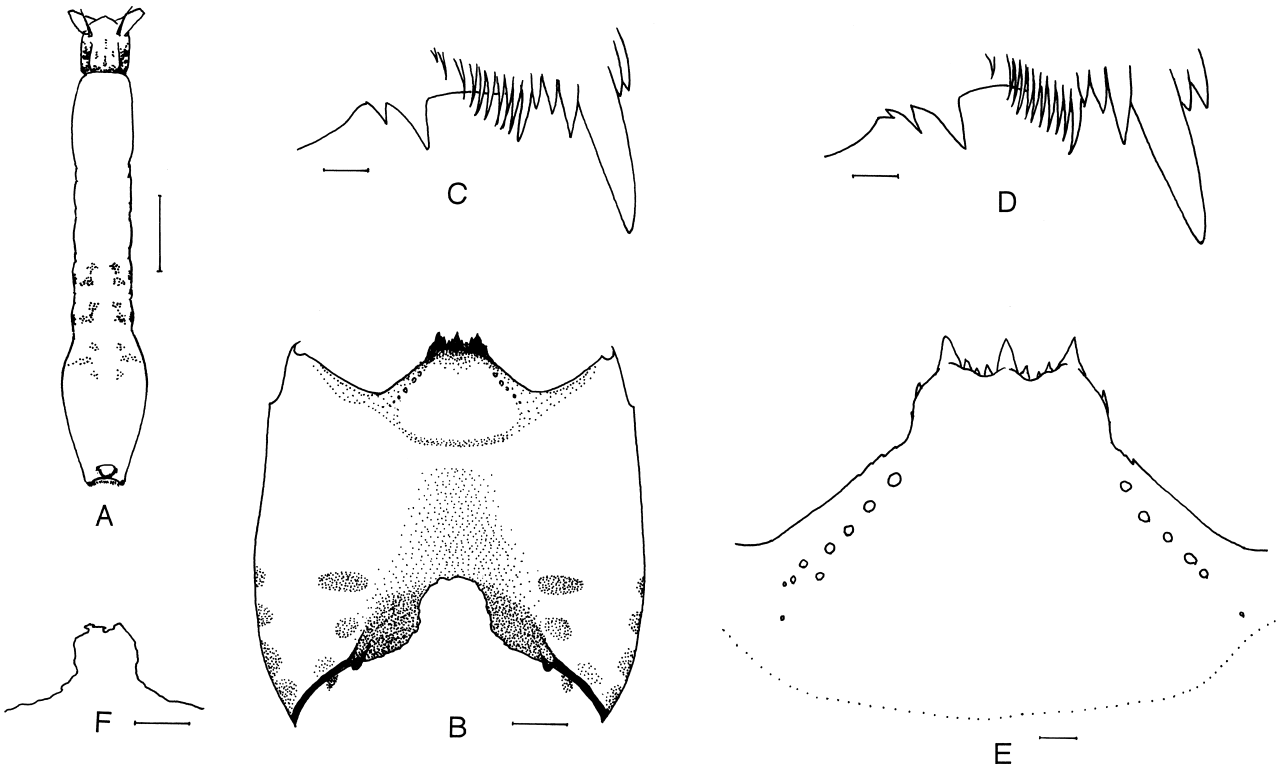

Fig. 4. Mature larva of *Simulium* (*Nevermannia*) *fruticosum* sp. nov. A, whole body (dorsal view); B, head capsule (ventral view); C, mandible; D, mandible with supernumerary serration; E, hypostomium; F, postgenal cleft. Scale bars. 1.0 mm for A; 0.1 mm for B and F; 0.02 mm for E; 0.01 mm for C and D.

on each side. Ventrally, segments 3−8 with comb-like groups of minute spines; segments 5−8 each with a pair of weakly-sclerotized light yellowish wide submedial sternal plates, on which comb-like groups of minute spines present; segment 4 nearly transparent, with 4 short slender simple setae on each side; segment 5 with a pair of bifid hooks submedially and a few short slender setae on each side; segments 6 and 7 each with 1 bifid hook submedially and 1 simple hook laterally, and a few slender setae on each side.

Cocoon (Fig. 3O). Simple, wall-pocket-shaped, compactly woven without open spaces in web, very thin, with anterior margin thickly woven, and extending ventrolaterally; individual threads invisible; 3.5−4.5 mm long by 2.5−3.6 mm wide.

Mature larva. Body length 6.2−6.5 mm. Body greyish yellow or light tawny, with well-defined colored markings (Fig. 4A); i.e., abdominal segments 3 and 4 each with 3 reddish-brown spots on each side, of which 2 spots lying submedially on dorsal surface (those spots very narrowly connected to each other), and 1 lying medially on lateral surface; abdominal segment 5 on each side with 1 reddish-brown spot submedially on anterior 1/2 of dorsal surface and 1 transverse similar colored band along its posterior margin; and abdominal segment 6 on each side with 1 reddish-brown spot submedially on anterior 1/2 of dorsal surface. Cephalic apotome yellow except posterior margin darkened, with distinct positive head spots; lateral surface of head capsule yellow, with distinct eyebrow containing dark spot; 2 large spots behind eye-spot region and 3 isolated spots below eye-spot region distinctively positive; ventral surface of head capsule (Fig. 4B) yellow (though postgenal bridge mostly darkened, with dark basal area on each side of postgenal cleft; horizontal and round spots on each side of postgenal cleft distinctively positive. Cervical sclerite composed of 2 small elliptical pieces, not fused to occiput, very widely separated medially from each other. Antenna consisting of 3 segments and apical sensillum, much longer than stem of labral fan; proportional lengths of 1st, 2nd, and 3rd segments 1.00 : 1.00−1.05 : 0.87−0.91. Labral fan with 26−29 main rays. Mandible (Fig. 4C) with mandibular serrations consisting of 2 teeth (1 large and 1 small); large tooth making nearly a right angle with mandible on apical side; supernumerary serrations absent (though 1 minute supernumerary tooth present on right mandible of 1 larva as shown in Fig. 4D); comb-teeth composed of 3 teeth, shortened from 1st to 3rd. Hypostomium (Fig. 4E)

with 9 apical teeth in row; median and corner teeth well developed; median tooth of 3 intermediate teeth on each side smallest; lateral serrations weakly developed apically; 6–9 hypostomal bristles lying slightly divergent posteriorly from lateral margin on each side. Postgenal cleft (Fig. 4B) small, 0.53–0.57 times as long as postgenal bridge, with anterior margin roughly rounded or irregularly defined (Fig. 4F). Thoracic cuticle bare. Abdominal cuticle bare except both sides of anal sclerite moderately covered with simple colorless setae, and lateral surface of last segment down to middle of ventral papilla also sparsely covered with similar but shorter setae. Rectal scales present. Rectal papilla compound, each of 3 lobes with 11 or 12 finger-like secondary lobules. Anal sclerite of usual X-form, with anterior arms slightly shorter than posterior ones, narrowly sclerotized along anterior arms; sensilla absent on and just posterior to basal juncture area; accessory sclerite absent. Last abdominal segment much expanded ventally forming large ventral papilla. Posterior circlet with about 86 rows of up to 16 hooklets per row.

TYPE SPECIMENS. Holotype female, reared from pupa, collected from a small stream slowly flowing among the bushes in a densely forested, leech-rich area (width 50 cm, depth ca. 2 cm, water temperature 25.0℃, completely shaded, altitude 1,420 m above sea level), Doi Pui, Chiang Mai province, Thailand, 21. V. 2005, by W. Choochote. Paratypes 4 females, 8 males, and 3 mature larvae, same data and date as those of holotype; 1 male, same data as those of holotype except date, 14. V. 2005; 1 pupa, same data as those of holotype except date, 16. IX. 2004, and water temperature 20.0℃.

BIOLOGICAL NOTES. The pupae and larvae of this new species were found on the surface of leaves and stalks of trailing grasses. Associated species were *S*. (*Gomphostilbia*) *inthanonense* Takaoka and Suzuki, *S*. (*Simulium*) *brevipar* Takaoka and Davies, *S*. (*S*.) *doipuiense* Takaoka and Choochote, *S*. (*S*.) *manooni* Takaoka and Choochote, and *S*. (*S*.) *mediocololatum* Takaoka and Choochote.

DISTRIBUTION. Thailand.

ETYMOLOGY. The species *fruticosum* refers to the stream running among the bushes where this new species was found. The Latin adjective *fruticosus* means bushy.

REMARKS. *Simulium* (*Nevermannia*) *fruticosum* sp. nov. is readily assigned to the *feuerborni* species-group by the combination of the following characters: male genitalia with a simple lamellate ventral plate, a short inwardly-twisted style, several parameral hooks, and a simple narrow median sclerite; pupal gill with six long thread-like filaments per side; and larval head with small short postgenal cleft.

Among the 16 known species of the *feuerborni* species-group [1, 6, 7], four species, i.e., *S*. (*N*.) *feuerborni*, *S*. (*N*.) *leigongshanense* Chen and Zhang from China, *S*. (*N*.) *praelargum* Datta from India, and *S*. (*N*.) *sasai* (Rubtsov) from Japan, have a cocoon with a distinct anterodorsal projection and thus differ from this new species. Eight other species of the same species-group have a simple cocoon but differ from this new species in the arrangement of gill filaments. Interestingly, *S*. (*N*.) *feuerborni* has the gill filaments arranged in a similar manner to those of *S*. (*N*.) *fruticosum* (i.e., four dorsal filaments arising close together and lying almost side by side horizontally as shown in Fig. 3 H–K).

The remaining four known species of the *feuerborni* species-group were described from adult males (and also females in one species) alone, their pupal and larval stages remaining unknown. Among these, *S*. (*N*.) *fuscinervis* Edwards recorded from Sabah [8] differs from the new species by having the paramere with 10 or 11 hooks; *S*. (*N*.) *bryopodium* Delfinado, described from Palawan Island, Philippines [9], also differs by the dark brown hind femora and ventral plate much depressed posteriorly; *S*. (*N*.) *senile* Brunetti, described from West Himalaya [10], is different by the style with no apical spine; *S*. (*N*.) *rufithorax* Brunetti, described from a male and four females collected from India [10], has a reddish-brown thorax according to the original description.

***Simulium* (*Nevermannia*) *chiangklangense* sp. nov.**

DESCRIPTION. **Female.** Body length 2.5 mm. Nearly the same as the female of *S*. (*N*.) *fruticosum* except for the following characteristics. ***Head.*** Frontal ratio 1.5:1.0:2.3. Frons-head ratio 1.0 : 4.8. Fronto-ocular area (Fig. 5A) somewhat shorter than that of *S*. (*N*.) *fruticosum*. Labrum 0.97 times as long as clypeus. Antenna with 1st flagellar segment about 1.6 times as long as 2nd one. Maxillary palp with proportional lengths of 3rd, 4th, and 5th segments 1.0: 1.0:1.6; 3rd segment (Fig. 5B) with elongate sensory vesicle 0.52 times as long as 3rd segment. Maxillary lacinia with 8 inner and 12 outer teeth. Mandible with 20 inner teeth but lacking outer teeth. ***Legs.*** Fore basitarsus 7.4 times as long as its greatest width. Hind basitarsus 6.6 times as long as wide, and 0.69 times and 0.61 times as wide as hind tibia and femur, respectively; calcipala well developed, nearly as long as wide, and 0.55 times as wide as greatest width of basitarsus. Claws (Fig. 5C) each with large basal tooth 0.43

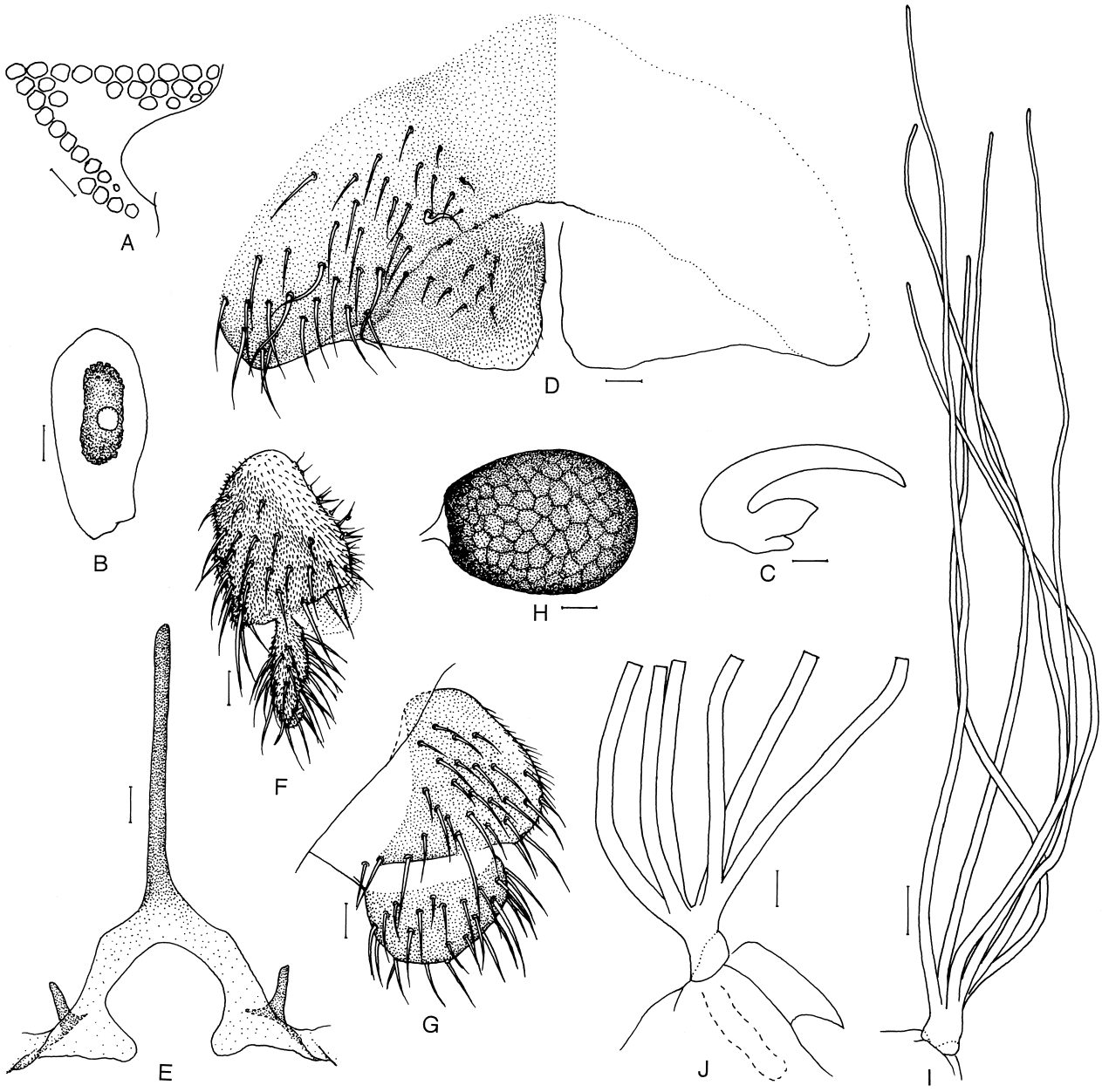

Fig. 5. Female and pupa of *Simulium* (*Nevermannia*) *chiangklangense* sp. nov. A, fronto-ocular area (right side); B, 3rd segment of maxillary palp with sensory vesicle (right side, front view); C, claw; D, 8th sternite and ovipositor valves *in situ* (ventral view); E, genital fork (ventral view); F and G, paraprocts and cerci (right side; F, ventral view; G, outer view); H, spermatheca; I, whole gill filaments (dorsal view); J, basal portion of gill filaments (outer view). Scale bars. 0.2 mm for I; 0.1 mm for J; 0.03 mm for A and B; 0.02 mm for D–H; 0.01 mm for C.

times as long as claw. ***Wing.*** Length 2.6 mm. ***Genitalia.*** Sternite 8 (Fig. 5D) wide, bare medially but furnished with 30–40 short and long hairs on each side. Ovipositor valves (Fig. 5D) with 9 or 10 short setae; inner margins gently undulate. Genital fork (Fig. 5E) with longer and narrower projection directed anteriorly on each arm than that of *S*. (*N*.) *fruticosum*. Paraproct in ventral view (Fig. 5F) somewhat wider than that of *S*. (*N*.) *fruticosum*; its anteroinner surface with 16 or 17 sensilla. Cercus in lateral view (Fig. 5G) about half as long as paraproct. Spermatheca (Fig. 5H) ovoidal, 1.3 times as long as its greatest width.

Male. Unknown.

Pupa. Body length 3.5 mm. Nearly the same as the pupa of *S*. (*N*.) *fruticosum* except for the following characteristics. ***Head.*** Integument moderately covered with round tubercles. ***Thorax.*** Gill (Fig. 5I,J) with 6 long thread-like slender filaments (length including basal common stalk 4.0−4.5 mm), of which 2 inner filaments arising independently, and 4 others arranged in 2 short-stalked pairs (1 ventroouter and 1 dorsal), all arising nearly at the same level from short basal common stalk; all filaments somewhat diverged basally from one another, then directed forwards; all filaments dark brown, tapered apically, subequal in length and thickness to one another, and longer than pupal body. ***Abdomen.*** Dorsal surfaces of all segments uniformly yellowish; minute tubercles on surfaces of segments 5−9 indistinct. ***Cocoon.*** 5.0 mm long by 3.2 mm wide.

Mature larva. Unknown.

TYPE SPECIMEN. Holotype female, reared from pupa, collected from a slow-flowing stream (width 0.3−0.5 m, depth 2−3 cm, water temperature 15.0℃, partially shaded, altitude 790 m above sea level) in a forested area, Chiang Klang, Nan province, northern Thailand, 2.XII. 2004, by W. Choochote.

BIOLOGICAL NOTES. The pupa of this new species was found on the surface of a leaf in the water. Associated species was *S*. (*G*.) *inthanonense*.

DISTRIBUTION. Thailand.

ETYMOLOGY. The species *chiangklangense* refers to the region, Chiang Klang, where this new species was found.

REMARKS. *Simulium* (*Nevermannia*) *chiangklangense* sp. nov. is also assigned to the *feuerborni* species-group by the female genitalia and pupal gill with six long thread-like filaments per side.

This new species is characterized by the simple cocoon and the pupal gill filaments arranged in two short-stalked pairs and two individual filaments (Fig. 5J). The combination of these two characteristics easily separates *S*. (*N*.) *chiangklangense* from other members of the *feuerborni* species-group.

The female of this new species is very similar in appearance to those of *S*. (*N*.) *feuerborni* [11] and *S*. (*N*.) *fruticosum* but appears to be distinguished from the latter two species by the longer and narrower projection directed anteriorly from each arm of the genital fork (Fig. 5E), and also from *S*. (*N*.) *fruticosum* by the shorter claw tooth (Fig. 5C).

There is a possibility that *S*. (*N*.) *chiangklangense* is conspecific to one of the three known species of the *feuerborni* species-group which were described from adult males alone (i.e., *S*. (*N*.) *fuscinervis*, *S*. (*N*.) *bryopodium*, and *S*. (*N*.) *senile*).

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THE EFFECT OF MEDICATION ON QUESTIONNAIRE ANALYSIS OF CHILDREN WITH *SCHISTOSOMA MANSONI* INFECTION IN TANZANIA

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Abstract: The effect of mass treatment on questionnaire results in the diagnosis of schistosomiasis mansoni was examined in 267 school children in an endemic area of Tanzania by Kato-Katz analysis of fecal specimens. The questionnaire asked for information about self-diagnosis, abdominal symptoms, blood in stools, history of wild water contact, stool examination and medication for schistosomiasis, and knowledge of the disease. A logistic regression analysis disclosed a significant association between schistosomiasis and "diarrhea" ($p = 0.007$; odds ratio, 32.0; confidence interval, 2.5 - 403.3) and "abdominal enlargement" ($p = 0.003$; odds ratio, 15.2; confidence interval, 2.6 - 90.1) among 61 children who had no history of medication for schistosomiasis. The sensitivity and specificity of the model were 86% and 64%, respectively. In contrast, no significant correlation was observed either for the 116 treated children, or for all the 267 children after the mass treatment. We conclude, therefore, that for children who had no history of medication for schistosomiasis, the questionnaire for abdominal manifestations provides reliable information on *S. mansoni* infection. However, once a child takes medication, the questionnaire becomes unreliable. This observation suggests that immunomodulation by anti-schistosomiasis drugs that kill adult worms exerts an effect on the appearance of abdominal manifestations and might explain the ambiguity of clinical symptoms in chronically infested patients, except in terminal cases. Further studies are required to develop a simple, rapid and cost-effective diagnostic method for monitoring *S. mansoni* infection after medication in local areas without resort to laboratory-based identification of schistosomiasis.
Key words: *Schistosoma mansoni*, self-diagnosis, selective mass treatment, Tanzania

INTRODUCTION

Schistosomiasis is one of the widespread parasitic diseases with important public health implications in sub-Saharan countries. Disease control depends mostly on medication treatment, because praziquantel, the drug of choice, is safe and relatively inexpensive [1]. Several approaches for community-based treatment have been developed, including mass treatment, selective population treatment and selective group treatment [2]. The main target population is school-age children between 5 and 15 years old, because they show the highest prevalence and intensity in the entire population [2, 3, 4] and because they are the main source of the transmission of infection. Following WHO recommendations, school-based mass treatment with praziquantel has been conducted in many high prevalence areas [5].

The strategy emphasizing school-based mass treatment, however, is not cost-effective in low prevalence areas. The prevalence usually declines to a low level after a series of mass treatments in high prevalence areas. Subsequently, the school-based mass treatment approach should be replaced

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Running title: Medication effect for *S. mansoni* infection on questionnaire

with selective treatment. The most reliable diagnostic method for *Schistosoma mansoni* (*S. mansoni*) infection is detection of eggs in feces, with a rapid and simple Kato-Katz thick smear stool examination being applied to this end. However, well-trained health personnel and microscopes are required for this laboratory diagnosis, and it is laborious, time-consuming, and expensive. Therefore, a similarly rapid and simple yet cost-effective individual diagnostic method is required. This kind of simple method has been developed for the screening of *Schistosoma haematobium* infection based on self-reported haematuria and self-diagnosis [6, 7, 8, 9]. Regarding *S. mansoni* infection, several morbidity studies have been carried out since the early 1970s, before the discovery of an effective medication (praziquantel). These studies found that *S. mansoni* frequently causes abdominal pain, blood in stool, bloody diarrhea, diarrhea in general, hepatomegaly, and splenomegaly [3, 10, 11, 12, 13, 14, 15, 16]. Based on theses studies, the diagnosis by questionnaire approach has been conducted for *S. mansoni* infection since 1985, initially focusing on subjective symptoms and the observation of stool appearance. But since studies reported low to moderate sensitivities and specificities [17, 18, 19], the questionnaires have been extended to include anamnestic information such as water-contact behavior [20, 21, 22, 23]. This approach, which was undertaken in several endemic areas, disclosed the potential of questionnaires for the identification of *S. mansoni* infection on the individual level [17, 18, 20, 22, 23, 24]. Supportive evidence for the application of a questionnaire for identification of *S. mansoni*, however, is still scarce, especially in the wake of mass treatment with praziquantel.

The purpose of this study was to evaluate the efficacy of questionnaires in the assessment of *S. mansoni* in one endemic area, with reference to the history of praziquantel medication in school-age children. An analysis was conducted to evaluate the potential of a questionnaire applied separately to children with and without a history of praziquantel medication.

MATERIALS AND METHODS

Cross sectional surveys on *S. mansoni* infection were conducted among Tanzanian children three times before and after mass treatment. The first questionnaire survey with stool examination was carried out in January and February 2001, and mass treatment was administered immediately after the survey. One month later, an additional stool examination for evaluation of efficacy of treatment was conducted. The second questionnaire survey with stool examination was carried out in February and March 2002, about one year after the treatment.

Area studied: The study area was Lower Moshi located in the southern part of the suburbs of Moshi Town in Kilimanjaro Region, Tanzania. The annual climate is divided into four periods: the long rainy season from March to June, cool dry season from July to October, short rainy season in November, and hot dry season from December to February. The main economic activity is wet-rice cultivation. The land is well developed for paddy cultivation with irrigation canals where water flows throughout the year. People usually cultivate crops three times a year. An agricultural development project supported by Japan International Cooperation Agency (JICA) has been conducted in this area since the 1970s.

Study population: The study was conducted in four primary schools; Mabogini, Rau River, Oria and Chekereni. There were 1,033 children registered in grades three and four in 2001.

Stool examination: A stool sample container was distributed to each child for collection of stool on the next day or the day after. Double 20 mg Kato-Katz thick smears [25] were processed and examined for the ova of *S. mansoni*, *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms by trained technicians and the authors. The number of eggs found in the samples was expressed as eggs per 1 gram (EPG) to reveal the intensity of infection. The presence of eggs was assessed for prevalence of infection. Geometric mean was calculated for expressing intensity of groups.

Questionnaire survey: The questionnaire consisted of five parts containing a total of 35 questions (see Tables 3 and 4 for the questionnaire contents). The first part focused on self-diagnosis, with the question “Do you think you have bilharzia (*Kichocho* in local Swahili language)?” The second part dealt with 18 self-reported clinical manifestations self-recognized during the last two weeks prior to the survey, including abdominal pain, diarrhea, constipation, abdominal enlargement, and blood in stool. The third part dealt with the history of contact with irrigation and river water including swimming, bathing, fishing (mainly net-fishing), washing clothes, fetching water, and other purposes. The fourth part dealt with anamnestic information: blood in stool in the past, experience of stool examination, and history of anti-schistosomiasis medication. The last part dealt with recognition and knowledge of schistosomiasis. The questionnaire was written in Swahili, the local language. Under the supervision of a Tanzanian doctor, a field assistant read out the questions to the children in groups of 20, and the children completed the forms individually. Children recorded their answers as ‘yes’, ‘no’ or ‘don’t

know'. We combined 'no' and 'don't know', and used the percentage of 'yes' replies to determine prevalence.

Treatment: Mass treatment with praziquantel was carried out in the schools under the supervision of a medical nurse in February 2001. Each child received 40 mg/kg of praziquantel. At the end of the study, the infected children were treated again. For the treatment of intestinal parasitic infection, we used mebendazole at a dose of 600 mg per person as a mass treatment in February 2001 and as a selective treatment in March 2002.

Analysis: The results of the study were analyzed using the SPSS® (version 11.5) software package. First, the prevalence of infestation by age and sex and by all the 35 variables in the questionnaire was calculated. Then the odds ratios (ORs) of infection were calculated and tested by Chi-square analysis. By using the variables correlated at less than the 0.10 level in univariate analyses, logistic regression analysis was conducted to assess the associations with infection after correcting for age and sex. The sensitivities and specificities of logistic regression models were calculated to assess the effectiveness of the questionnaire as a tool for individual diagnosis of infection. The analysis was conducted separately for those who had received treatment before the mass treatment and for those who had never received the treatment.

Ethical guidelines: This study was part of an epidemiological survey permitted by the National Institute of Medical Research, Ministry of Health, United Republic of Tanzania. We started the program by explaining the outline of the study to local authorities. Then we explained orally the purpose and methods of the study to the school children, their parents and teachers at school assemblies. At mass treatment, we treated all children who desired treatment irrespective of enrollment in the study. We treated infected children again for *S. mansoni* and other helminthes at the end of the study in March 2002. Informed consent was not obtained in written form.

RESULTS

Number of subjects for analysis: Out of 1,033 children registered in grades 3 and 4 in the 4 schools, demographic data such as sex and age were obtained from 830 children (417 males and 413 females, aged 7 to 17). Out of these 830 children, 267 (32.2%) had undergone three stool examinations, mass treatment after the first survey, and two questionnaires. The present analysis is restricted to these 267 children. The number of children by age and sex is shown in Table 1 with prevalence of *S. mansoni* infection. No significant difference in age or sex were observed between the 267 children and the rest of the 830 children.

Prevalence and the intensity of *S. mansoni* infestation: Among the 267 children, the prevalence of schistosomiasis was 67.4% and the mean intensity of schistosoma eggs in feces was 29.9/g at the first stool examination prior to mass treatment (Figure 1). The prevalence and intensity were not significantly different either by sex or by age. The prevalence decreased to 6.4% (McNemar test, $p < 0.001$) and the mean intensity to 0.3/g (t-test, $p < 0.001$) one month after the mass treatment (2nd fecal analysis). However, the prevalence increased to 27.0% (McNemar test, $p < 0.001$) with a slight increase in the mean intensity to 2.5/g (t-test, $p < 0.001$) one year after the treatment (3rd fecal analysis), although this prevalence and intensity were still significantly lower than those prior to mass treatment (McNemar test $p < 0.001$, t-test $p < 0.001$, respectively). The mean EPG calculated from only positive samples decreased from 160.8/g ($n = 180$) to 74.8/g ($n = 17$) by mass treatment and then increased to 105.2/g ($n = 72$) one year after the treatment. The prevalence of heavy infection (more than 400 EPG) de-

Table 1 The number of children and prevalence of *S. mansoni* infection before mass-treatment by age and sex

	Boys			Girls		
Age	Examined	Infected	Prevalence (%)	Examined	Infected	Prevalence (%)
7-9	9	7	(77.8)	17	11	(64.7)
10	30	20	(66.7)	29	21	(72.4)
11	41	29	(70.7)	50	31	(62.0)
12	32	23	(71.9)	19	9	(47.4)
13	15	10	(66.7)	10	7	(70.0)
14-17	11	9	(81.8)	4	3	(75.0)
Sub-total	138	98	(71.0)	129	82	(63.6)

In total, 267 children were examined and 180 (67.4%) were egg-positive.
No significant difference was found between sex or age.

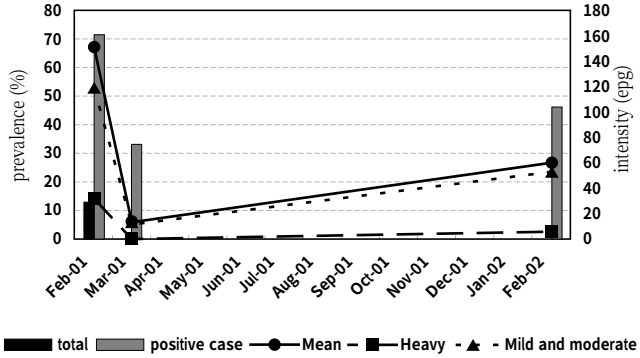

Figure 1 Prevalence and intensity of *S. mansoni* infection before, one month after, and one year after mass treatment. Solid line with circles: mean prevalence of infection; Dotted line with triangles: prevalence of mild and moderate infection (100 EPG or more); Dashed line with square: prevalence of heavy infection (400 EPG or more); Black bars: mean intensity (geometric mean EPG for the 267 children); Gray bars: mean intensity of positive cases (geometric mean EPG of 180, 17, 72 children, respectively).

creased from 14.2% to 0.4% by treatment, then increased to 3.0% after one year.

Experience of treatment: Of the 267 children, 61 had no history of medication for schistosomiasis before the mass treatment, while 116 children had a medication history. The remaining 90 children were not sure either way. The prevalence and intensity of infection did not differ significantly between non-treated and treated children (59.0% and 17.6/g for the former and 62.9% and 21.4/g for the latter) in the first stool examination.

Prevalence of water contact activities: About 90% of children reported having contact with irrigation/river water for the purpose of swimming/playing, bathing, crossing water and washing clothes, and fetching water in the first survey. About 40% of children had contacted water for the purpose of fishing in the first survey. The percentage of children contacting irrigation/river water in the second survey decreased slightly from the first survey in all activities.

Results of questionnaire survey in relation to egg detection before mass treatment for the 267 children: Before mass treatment, the most common symptom was “abdominal pain” (60%) followed by “headache” (52%) and “abdominal discomfort” (40%). But, no manifestation showed a significant increase with the level of intensity (Table 2). “Constipation” and “net-fishing” showed a significant correlation with infection (both $p < 0.05$) and “diarrhea” and “history of blood in stool” showed borderline significance (both, $p < 0.10$, by Chi-square test, Tables 3 and 4). For a

Table 2 Prevalence of signs and symptoms by the level of intensity of *S. mansoni* infection before mass-treatment

Manifestations	Percentage of “yes” answers in the questionnaire					Somers’ d	p
	Total	Level of intensity §					
		Negative	Mild	Moderate	Heavy		
		0 epg	1 - 99 epg	100 - 399epg	400 epg<		
	(n=267)	(n=87)	(n=51)	(n=91)	(n=38)		
abdominal pain	59.6	52.9	62.7	61.5	65.8	0.149	0.459
headache	52.4	46.0	51.0	58.2	55.3	0.130	0.415
abdominal discomfort	40.4	33.3	43.1	42.9	47.4	0.109	0.398
easy to be tired	28.8	25.3	31.4	29.7	31.6	0.451	0.834
fever	24.3	20.7	31.4	28.6	13.2	0.863	0.141
loss of appetite	20.6	20.7	27.5	16.5	21.1	0.609	0.491
cough	19.9	19.5	23.5	22.0	10.5	0.475	0.428
nausea/vomiting	18.4	16.1	21.6	20.9	13.2	0.943	0.632
constipation	17.2	10.3	27.5	19.8	13.2	0.371	0.058
diarrhea	16.5	10.3	19.6	18.7	21.1	0.085	0.303
fatigue	14.2	10.3	21.6	14.3	13.2	0.646	0.339
abdominal enlargement	14.2	9.2	19.6	17.6	10.5	0.391	0.233
dizziness/lassitude	12.4	10.3	13.7	12.1	15.8	0.482	0.845
skin rash	10.1	10.3	11.8	9.9	7.9	0.686	0.945
blood in stool	5.2	3.4	5.9	5.5	7.9	0.335	0.330
itching skin	4.5	3.4	3.9	4.4	7.9	0.383	0.348
weight loss	4.1	2.3	5.9	6.6	0.0	0.753	0.840
asthma	3.7	3.4	5.9	4.4	0.0	0.477	0.549

§ Intensity is categorized according to the definitions of WHO

Table 3 Prevalence of self-diagnosis and 2-week-recognition of symptoms before and after treatment, and for non-treated and treated and the odds ratio between infected and non-infected

Self-diagnosis and recognition of symptoms within two weeks	Percentage of "yes" answers in the questionnaire (%)				Odds ratio and its 95% confidence interval			
	Before treatment			After treatment	Before treatment			After treatment
	all (n=267)	never treated (n=61)	treated (n=116)	all (n=267)	all (n=267)	never treated (n=61)	treated (n=116)	all (n=267)
self-diagnosis	7.7	6.7	9.0	25.8	0.90 0.314-2.586	2.09 0.204-21.382	0.53 0.144-1.957	1.15 0.623-2.107
loss of appetite	20.6	44.3	19.8	43.8	0.99 0.527-1.867	2.38 0.819-6.890	1.13 0.435-2.941	1.12 0.649-1.925
nausea/vomiting	18.4	27.9	25.0	61.4	1.26 0.637-2.486	0.99 0.317-3.085	3.72* 1.299-10.665	1.06 0.610-1.858
abdominal enlargement	14.2	29.5	5.2	7.1	1.98 0.865-4.512	9.20* 1.881-44.998	0.57 0.110-2.966	2.09 0.805-5.429
abdominal discomfort	40.4	62.3	29.3	71.5	1.56 0.917-2.669	2.82 § 0.965-8.222	1.97 0.817-4.741	0.80 0.442-1.431
abdominal pain	59.6	72.1	43.1	85.4	1.50 0.895-2.524	1.97 0.634-6.115	1.26 0.587-2.713	1.82 0.766-4.338
diarrhea	16.5	19.7	25.9	49.4	2.09 § 0.957-4.575	10.56* 1.265-88.184	1.89 0.755-4.719	1.91* 1.101-3.316
constipation	17.2	36.1	10.3	38.2	2.24* 1.029-4.887	5.25* 1.500-18.380	1.88 0.479-7.342	0.96 0.550-1.677
blood in stool	5.2	8.2	6.0	30.5	1.82 0.495-6.708	3.00 0.315-28.586	3.76 0.437-32.349	1.31 0.737-2.332
easy to be tired	28.8	57.4	25.9	41.2	1.30 0.729-2.318	2.55 § 0.890-7.279	1.89 0.755-4.719	0.88 0.505-1.524
fatigue	14.2	24.6	17.2	63.7	1.66 0.751-3.690	2.31 0.640-8.333	2.74 § 0.850-8.808	1.30 0.734-2.312
fever	24.3	45.9	26.7	58.1	1.35 0.731-2.509	1.50 0.534-4.214	2.52 § 0.979-6.484	1.29 0.740-2.243
cough	19.9	37.7	19.8	58.6	1.03 0.541-1.959	1.52 0.521-4.426	1.44 0.541-3.851	1.59 0.904-2.805
asthma	3.7	9.7	3.4	16.1	1.13 0.286-4.492	1.44 0.242-8.524	1.80 0.181-17.869	1.21 0.593-2.479
weight loss	4.1	4.9	4.3	32.2	2.24 0.473-10.582	1.41 0.121-16.470	2.43 0.263-22.522	0.75 0.415-1.363
dizziness/lassitude	12.4	23.0	14.7	50.2	1.33 0.591-3.006	1.33 0.387-4.592	3.16 § 0.854-11.725	1.07 0.622-1.834
skin rash	10.1	18.0	12.1	30.3	0.96 0.414-2.241	0.80 0.215-2.979	2.37 0.621-9.008	1.85* 1.051-3.273
headache	52.4	68.9	38.8	83.5	1.47 0.878-2.456	0.78 0.255-2.371	2.54* 1.111-5.788	1.13 0.537-2.375
itching skin	4.5	3.3	8.6	43.8	1.47 0.389-5.586	0.69 0.041-11.505	2.52 0.510-12.472	1.12 0.649-1.925

*: significant at 0.05 level
§ : borderline significant at 0.1 level

binary logistic regression analysis using "constipation", "net fishing", "history of blood in stool", "diarrhea", age, and sex as independent variables, only "constipation" (odds ratio, OR = 2.27, confidence interval, CI = 1.04 - 4.96) was positively associated with infection (Table 5a). The prediction of the model, however, was not significant.

Results of questionnaire survey in relation to egg detection at one year after mass treatment for the 267 children: Results: "Abdominal pain", "headache" and "abdominal discomfort" remained common at one year after the treatment. The prevalence of all signs and symptoms except "abdominal enlargement" increased from the first questionnaire (Table 3). The prevalence of "abdominal enlargement" decreased from 14% before treatment to 7% at one year after treatment. The prevalence of self-diagnosis of *S. mansoni* infection was 8% before the treatment and increased to 26% one year after the treatment.

"Diarrhea" and "skin rash" were significantly associated with schistosomiasis (both $p < 0.05$), and "bathing" showed a borderline significant association with prevalence ($p=0.09$). For a binary logistic regression analysis using "diarrhea", "skin rash", "bathing", age, and sex as independent variables, only "diarrhea" (OR = 1.96, CI = 1.13 -

Table 4 Questionnaire of history of wild water contact, knowledge and recognition, experience of fecal examination, fecal blood hisotry and treatment before and after treatment, and for non-treated and treated and the odds ratio between infected and non-infected

	Proportion of answering "yes" (%)				Odds ratio and its 95% confidence interval			
	Before treatment			After treatment	Before treatment			After treatment
	all (n=267)	never treated (n=61)	treated (n=116)	all (n=267)	all (n=267)	never treated (n=61)	treated (n=116)	all (n=267)
Water contact activities								
any purpose	91.0	96.7	87.1	83.7	0.39 0.128-1.165	1.46 0.087-24.468	0.23* 0.048-1.051	1.63 0.716-3.733
swimming/playing	90.5	88.1	87.5	53.6	0.80 0.317-2.004	1.11 0.224-5.463	0.62 0.178-2.162	1.12 0.649-1.922
bathing	91.7	91.5	89.4	49.8	0.77 0.289-2.074	2.36 0.363-15.309	0.52 0.129-2.083	1.60 § 0.926-2.764
fishing	38.7	22.0	10.6	17.6	1.84* 1.056-3.220	0.75 0.217-2.594	0.79 0.225-2.788	1.34 0.677-2.655
crossing the water	92.9	94.9	85.6	36.7	0.99 0.357-2.729	0.72 0.061-8.387	0.70 0.222-2.227	1.44 0.832-2.509
washing clothes	94.1	96.6	87.5	59.2	0.70 0.218-2.283	1.48 0.088-24.847	0.40 0.103-1.551	1.55 0.878-2.722
fetching water	88.5	84.7	89.4	54.3	0.88 0.381-2.018	1.20 0.287-5.021	0.83 0.226-3.023	1.25 0.724-2.160
for agricultural purpose	87.7	84.7	84.6	55.8	1.10 0.501-2.416	1.20 0.287-5.021	0.44 0.131-1.467	1.07 0.618-1.838
for animal husbandry	78.3	67.8	75.0	29.2	0.77 0.400-1.471	0.26* 0.075-0.945	0.58 0.223-1.480	0.83 0.451-1.516
Anamnestic information								
Have you ever had blood in stool?	23.0	23.0	28.7	no data	2.08 § 0.985-4.392	5.75* 1.158-28.551	1.94 0.769-4.883	no data
Have you ever taken medication for schistosomiasis?	60.7	0	100.0	72.2	1.07 0.588-1.947	(-) (-)	1.00 (-)	1.30 0.697-2.438
Have you ever taken fecal examination?	83.9	50.8	No data	95.5	1.22 0.556-2.656	0.92 0.333-2.562	(-) (-)	1.11 0.293-4.231
Recognition and knowledge on the disease								
Have you ever heard of disease of bilharzia?	83.1	50.8	87.8	79.6	1.50 0.774-2.906	2.79 § 0.971-8.034	0.11* 0.014-0.894	1.20 0.598-2.391
Do you know what bilharzia is?	68.4	23.0	78.3	47.9	1.07 0.617-1.860	2.02 0.553-7.367	0.18* 0.050-0.639	1.17 0.676-2.014
Contacting with river water causes bilharzia?	34.7	16.9	43.9	16.1	1.22 0.702-2.116	(-) (-)	0.84 0.393-1.803	0.79 0.368-1.703
Drinking river water causes bilharzia?	22.4	15.3	36.8	62.2	1.11 0.592-2.084	(-) (-)	0.98 0.445-2.137	1.11 0.631-1.937

*: significant at 0.05 level

§ : borderline significant at 0.1 level

3.43) was positively associated with infection (Table 5d). The prediction of the model, however, was not significant. The level of intensity of infection was also related with "diarrhea", increasing from 45% of negative children, to 52% of lightly infected children, to 66% of moderately infected children and to 75% of heavily infected children (Somers'd test, $p < 0.05$, data not shown).

Results of questionnaire survey in relation to egg detection before mass treatment for the 61 non-treated and 116 treated children: Next, we compared the 61 non-treated and 116 treated children. In children without a history of medication for schistosomiasis, "diarrhea", "constipation", "abdominal enlargement", and "recognition of blood in stool" were significantly associated with schistosomiasis by univariate analyses. "Abdominal discomfort", "easy to be tired", and "recognition of the disease (yes to the question "Have you ever heard of bilharzia?")" were associated with borderline significance in these children (Tables 3 and 4). Recognition of blood in stool over the past two weeks was not significant, but a history of this recognition was significant in the non-treated children.

Among the 116 children with a medication history, meanwhile, "nausea/vomiting", "headache", recognition of the disease, and knowledge of the disease (yes to the question "Do you know what bilharzia is?") were significant,

Table 5 Logistic regression of *S. mansoni* infection with questionnaire's items

a. Before mass-chemotherapy (all children, n = 267)

Variable	Parameter estimate	Standard error	p	Odds ratio	95.0% C.I † †	
					Lower	Upper
Constipation	0.820	0.399	0.040	2.271	1.039	4.961
Constant	0.822	1.106	0.458	2.274		

Sensitivity = 100%, Specificity = 0%

b. Before mass-chemotherapy, (never-treated children, n = 61)

Variable	Parameter estimate	Standard error	p	Odds ratio	95.0% C.I. † †	
					Lower	Upper
Diarrhea	3.466	1.293	0.007	31.993	2.538	403.342
Abdominal enlargement	2.719	0.909	0.003	15.166	2.553	90.080
Constant	-7.923	3.582	0.027	0.000		

Sensitivity = 86.1%, Specificity = 64%

c. Before mass-chemotherapy, (ever-treated children, n = 116)

	Parameter estimate	Standard error	P	Odds ratio	95.0% C.I. † †	
					Lower	Upper
Recognition of the disease	-1.671	0.655	0.011	0.188	0.052	0.679
Constant	2.862	2.001	0.153	17.488		

Sensitivity = 100%, Specificity = 2.4%

d. After mass-chemotherapy, (all children, n = 267)

	Parameter estimate	Standard error	p	Odds ratio	95.0% C.I. † †	
					Lower	Upper
Diarrhea	0.675	0.284	0.017	1.964	1.126	3.425
Constant	-2.232	1.193	0.061	0.107		

Sensitivity = 0%, Specificity = 100%

but abdominal manifestation was not significant (Tables 3 and 4). "Fever", "dizziness", "fatigue" and "contact with river water for any purpose" showed borderline significance.

We examined the effect of treatment on selected manifestations using binary logistic regressions explaining *S. mansoni* infection for the 61 non-treated children (Table 5b) and for the 116 treated children (Table 5c). In the non-treated children, "diarrhea" (p = 0.007, OR = 32.0, CI = 2.54 - 403.3) and "abdominal enlargement" (p = 0.003, OR = 15.2, CI = 2.55 - 90.1) were significantly associated with infection, and the model prediction was significant (Chi-square = 26.98, p < 0.001). The sensitivity and the specificity of the logistic predictive model were 86.1% and 64.0%, respectively.

In the treated children, only knowledge of the disease was significantly and negatively associated with prevalence, and the prediction was significant (the model Chi-square = 10.085, p = 0.018) (Table 5c). But, the specificity of the model prediction was very low (2.4%), while the sensitivity was 100%.

Other parasitic infections: The prevalence of eggs of *A. lumbricoides*, *T. trichiura a*nd hookworm was 6.7%, 10.9%, and 9.0% respectively in the first examination. It declined to 5.3%, 4.9% and 4.1% respectively one year after mass treatment. At the first survey, *A. lumbricoides* infection was correlated with *S. mansoni* infection (Chi-square = 9.329, p = 0.002), but it was not significantly associated with any sign or symptom of *S. mansoni* infection. Hookworm infection was correlated with "constipation". However, it did not correlate with *S. mansoni* infection. *T. trichiura* infection showed no correlation with *S. mansoni* infection or with any sign or symptom.

DISCUSSION

The present study was conducted in a high endemic area according to the WHO definition [4], the prevalence of schistosomiasis being 67.4% among school children aged from 7 to 17 years old. The results indicated that subjective abdominal symptoms are related with infection of *S. mansoni* and that questionnaire on the subjective abdominal symptoms can be used for the screening of *S. mansoni* infection in children who have never undergone treatment for *S. mansoni* infection. "Diarrhea" and "abdominal enlargement" correlated well with infection when the children had

no history of treatment. These correlations were not seen in children who had undergone treatment in the past, although the prevalence and intensity of infection did not differ from those of the non-treated children. At one year after mass treatment, no suitable model for the prediction of *S. mansoni* infection was obtained by using self-diagnosis, subjective symptoms, water contact behavior, anamnestic information, or recognition and knowledge of the disease.

S. mansoni causes a chronic disease with various stages and many symptoms and signs at each stage, adult worms being present in the mesenteric veins and eggs in various organs including the liver, lungs and gastrointestinal tract. Deposition of eggs in the alimentary tract causes mucosal lesions frequently associated with hemorrhage resulting in blood in the stool. The abdominal symptoms might be influenced by adult worms in the mesenteric veins and egg embolism scattered from the adult female worms. The human body responds to schistosomula during acute infection or when eggs are retained in tissue [26]. Regarding adult worms, no response has been observed around the live worms. Colley advocated the hypothesis that praziquantel treatment alters host-parasite relationships, leading to multiple immunologic changes through the killing of adult worms. The antigens released from dead adult worms cause many immune responses and produce some resistance to reinfection [27]. This hypothesis is supported by an animal experiment using immuno-compenent and deficient mice [28]. B cell deficient mice showed a delayed response after praziquantel treatment, suggesting that the immunological responses are required for the killing of adult worms during treatment. Taken together, this evidence and our results suggest that the abdominal manifestations become obvious in the host who has recently been infected and not treated by medication.

The association between intensity of infection and morbidity was frequently reported. It was shown that complaints of “abdominal pain” and “blood in stool” correlated significantly with *S. mansoni* egg counts in a high prevalence village [11]. Heavily infected individuals frequently complained of “abdominal pain” [14]. Our study, however, did not confirm any correlation between intensity of infection and the prevalence of abdominal symptoms. The reason for this is not clear. “Abdominal discomfort”, “diarrhea”, “constipation”, “easy to be tired” and “abdominal enlargement” were associated with the level of intensity of infection (Somers’d test, $p = 0.021$, 0.003, 0.004, 0.038 and < 0.001, respectively) in the non-treated children. Almost all of these children had abdominal manifestations. On the other hand, “dizziness/lassitude”, “nausea/ vomiting” and “headache” were associated with the level of intensity of infection (Somers’d test, $p = 0.030$, 0.014 and 0.018, respectively) in the treated children.

One of the limitations of the present study is that it examined only the ova of helminthes in fecal specimens and did not determine other causes of “diarrhea” and “abdominal enlargement” such as protozoan or bacterial intestinal infection. Since abdominal manifestations are non-specific, it is impossible to overrule other causes. In fact our results show that the infection of hookworm causes “constipation” as an abdominal symptom, although the prevalence was not high. The intestinal parasitic infestation did not contribute significantly to the abdominal manifestations in our study because of its low prevalence, although the hookworm infection was significant as a cause of “constipation”. From this viewpoint, the abdominal manifestation should be applied while ruling out other intestinal infections and infestations.

“Blood in stool” is well documented as the most notable subjective sign of *S. mansoni* infection. Sukuwa (1985) and Proietti (1989) reported that “blood in stool” had the highest specificity (94.9%) [17] and the highest positive predictive value (38.9%) [18] among the symptoms. In the present study, however, “recognition of blood in stool” did not prove to be a specific sign of infection. With regard to the individual perception of clinical manifestations, the only reliable variables identified were “diarrhea” and “abdominal enlargement”. The reason may be that our study population was limited to school children while the previous studies included adults.

In the variables of risk factors such as self-diagnosis, water contact history, anamnestic information, and recognition and knowledge of the disease, we did not identify any significant variable that could be used for individual diagnosis. The use of risk factors has been reported to be limited in its potential to identify the infection of *S. mansoni*. Utzinger (2000) argued that recalled water-contact patterns collected by questionnaire might be useful for self-diagnosis of schistosomiasis, showing sensitivity and specificity of “crossing the river” to be 61-70% and 47-52%, respectively, and those of “swimming/bathing” to be 77-81% and 37-68% respectively [23]. According to Brooker (2001), the reported “swimming” had 80% sensitivity and 83% specificity [21]. However, Lima e Costa (1998) pointed out that sociodemographic variables and water contact activities associated with *S. mansoni* infection differed among the study areas, thus their validity for diagnosis of infection similarly differed among the areas [22]. Unlike previous studies, water contact behavior was not correlated with infection in our study. The reason might be that we did not define the period of the behaviors in the questionnaire and therefore that many children reported having contact with the irrigation/river water.

After a series of mass treatments in high prevalence areas, prevalence will decrease considerably. Subsequently, the school-based mass treatment approach might be replaced by selective treatment, and children can be monitored for re-infection individually. But as the present study showed, diagnosis by questionnaire becomes inapplicable once children take medication. Although stool examination is the most reliable method for individual identification of *S. mansoni* infection in an area of low intensity infection, other simple, rapid and cost-effective individual diagnostic methods are still required in areas where laboratory examination has not been established. Moreover, it is imperative that old-fashioned questionnaire analysis be analyzed for usefulness for schistosomiasis using praziquantel-resistant clone [29].

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